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(54) Selectively O-acylated glycosaminoglycans

(57) The glycosaminoglycans of the invention are selectively O-acylated in a modulable manner on their –OH free groups, the carboxylic or aminated functional groups are not altered.

These compounds have a very long-lasting pharmacological activity.

EP 0 356 275 A1

Description

SELECTIVELY O-ACETYL GLYCOSAMINOGLYCANS

The invention concerns selectively O-acetylated glycosaminoglycans, their preparation process and the pharmaceutical compositions containing them and their use in therapeutics.

The term "glycosaminoglycan" is understood as a substance constituted of uronic acid bonds (D-glucuronic acid or L-iduronic acid) and aminated sugars, the latter being glucosamines or galactosamines.

Glycosaminoglycans of natural origin are constituted by more or less homogeneous mixtures of chains of disaccharide units formed by a uronic subunit (glucuronic acid or acid iduronic) and by a o-amine subunit (glucosamine or galactosamine) joined by a bond 1 - >4 or 1 - >3.

In glycosaminoglycan, hydroxyl clusters are variously replaced by functional clusters, in particular sulfate groups, aminated groups of osamines are replaced by sulfate and/or acetyl groups.

The glycosaminoglycans in question here include 6 types of products: heparin, heparan, sulfate, A sulfate chondroitin, C sulfate chondroitin, B sulfate chondroitin, more specifically called sulfate dermatan and hyaluronic acid. They are characterized by the following (A) and (B) disaccharide structures:

(A)

wherein R is a SO_3- group and/or an acetyl group and the sign (]) indicates that the carboxyl group can be below the cycle plane (iduronic unit) or above the cycle plane (glucuronic unit) and

(B)

Glycosaminoglycans with an (A) base structure herein above are called heparin-type glycosaminoglycan (or GAGs) and comprise sulfate heparins and heparans and glycosaminoglycans with the (B)-base structure herein above are called sulfate chondroitin-type GAGs and comprise A and C sulfate chondroitins and sulfate dermatan.

Hyaluronic acid has the (B) base structure wherein the galactosamine is replaced by a glucosamine.

As mentioned hereinabove, heparin-type GAGs and sulfate chondroitin-type GAGs both have a more or less high percentage of hydroxyl groups of n disaccharide units present in the form of sulfuric acid esters.

Thus, the heparin can be represented by the (A) base structure above, wherein n can vary from 1 to 80, R is in a majority of the n units as an SO_3- group and, in the remainder of the cases, an acetyl group, the OH groups in position 8 of the glucosamine and in position 2 of the uronic acid are in the majority of cases sulfates, whereas the OH group in position 3 of the glucosamine is only sulfated in a minority of cases; the OH in position 3 of the uronic acid is practically not sulfated, said uronic acid is in the majority of cases an iduronic acid.

The products with the (A) base structure also include the N-disulfated N-acetylated heparin that can be prepared as described in Nagasawa K and Inoue Y., Methods in Carbohydrate Chemistry, Academic Press, 1980, Vol. 8, pp. 291-294. This heparin is represented by the formula (A) in which R is an acetyl group.

The heparin also contains cases with the (A) structure, with an n varying from 3 to 15 and the same chemical profile. Said cases can be isolated by splitting according to known methods (e.g., US 4,692,436) and are called heparins of low molecular weight (lmws).

Lmw heparins, with the molecular distribution and the biological properties essentially identical to those of lmw heparins obtained by splitting, have been prepared by means of splitting heparin according to the following methods:
- nitrous depolymerization and reduction method (e.g., EP 37 319 of US 4,686,288) that cuts the molecule by attacking the N-sulfate glucosamine subunits to give a 2,5-anhydromanitol ending

A')

possibly sulfated on the primary hydroxyl in position 6;

- enzymatic depolymerization method (e.g., EP 244 235 and 244 238) or alkaline (e.g., EP 40 144) that cuts the molecule by attacking the uronic subunits to give a non-saturated α-β uronic acid ending

(A')

possibly 2-sulfated;
- oxidative depolymerization method (e.g., US 4,281,108 and 3P 121067) that cuts the molecule by oxidation while keeping the "natural" endings.

Other lmw heparins that have a molecular distribution other from that of natural heparin and specific biological properties can be prepared by periodical oxidation of the natural heparin followed by depolymerization by β-elimination or acid hydrolysis. Periodical oxidation cuts the non-sulfated uronic acid patterns between the carbons in position 2 and 3. It is known that such patterns are present in the bonding site with antithrombin III (AT III), plasmatic inhibitor of various serine-proteases of coagulation, and whose activity is significantly increased by the heparin acting as a cofactor. The periodical oxidation therefore enables the obtaining of products devoid of the bonding site with AT III and therefore essentially devoid of anticoagulant activity.

Lmw heparins of this type can be obtained in particular by implementing the process including the following stages:
- the oxidation provided of the heparin implemented by having the heparin react in aqueous solution with a final concentration of 0.5 to 5% (w/v) with a periodic acid salt at a final concentration of 0.5 to 1% (w/v) with a pH between 4.5 and 6.5 and preferably at a pH of 5, at a temperature from 0 to 10°C, for around 15 to 48 hours, sheltered from light;
- the depolymerization of heparin chains obtained by adding a strong base such as sodium, to a pH approximately higher than 11, in particular between 11 and 12, advantageously from 11.2 to 11.6, preferably around 11.5;
- the reduction of depolymerization fragments with the help of a reducing agent, and after elimination, if applicable, of the non-reacting reducing agent,
- the recovery of reduced heparin fragments, by precipitation with the help of a solvent in which they are insoluble;
- the isolation of fragments found by splitting with the help of alcohol, in the presence of a mineral salt, of an aqueous solution obtaining by placing in solution in water the previously isolated precipitate and by recovering the precipitate formed.

These lmw heparins respond to the following formula (C):

(C)

wherein n can vary from 8 to 14, R is at least around 90% of the n units [in] a group S03- group and, in the remainder of the cases, an acetyl group, OH groups in position 3 of the glucosamine can be sulfated and OH groups in position 6 of the glucosamine being sulfated in 70% of the cases. Moreover, due to 1 pattern for 2 chains at least of this lmw heparin, the iduronic acid is replaced by an open non-sulfated uronic acid pattern (D-glucuronic or L-iduronic) between the carbons in position 2 and 3 of the following formula:

[formula]

Such lmw heparins can still be split by gel filtration according to known techniques in order to obtain mixtures of homogeneous fragments with regard to their molecular mass, devoid of the bonding site at AT III.

Other heparin-type glycosaminoglycans are constituted by formula (A) or (C) GAGs, wherein the carboxylic function brought about by the uronic acid is esterized, for example by condensation of an alcohol in the presence of a carbodiimide-type condensation agent, as described in patent FR no. 2 159 724 or else by alkylation of the carboxyl with the help of an alkyl halogen in the presence of a weak base.

Similarly, sulfate chondroitin-type glycosaminoglycans are constituted by formula (B) glycosaminoglycans wherein carboxylic functions of the uronic acids are esterized, for example by alkylation of the carboxyl with the help of an alkyl halogen in the presence of a weak base.

Obtaining such esters for the hyaluronic acid is described in patent EP No. 215 453 by processing the hyaluronic acid in the form of quaternary ammonium salt by an alcohol in the presence of a catalyst or by an etherifying agent.

The sulfate heparin is represented by formula (A) herein above, wherein n can vary from 1 to 80, R represents in the majority of the cases an acetyl group and in a minority of the cases SO_3-. Furthermore, in the great majority of the cases, the OH groups in position B of the glucosamine are sulfates, uronic acid is in the majority of cases glucuronic acid.

The A and C sulfate chondroitins are represented by the formula (B) herein above wherein n can very from 1 to 80 and respectively, 4-OH and 6-OH groups of the glucosamine are sulfated, the uronic acid is in the majority of the cases a glucuronic acid.

The sulfate dermatan has a structure that is substantially identical to that of the A sulfate chondroitin, but the uronic acid subunit is an iduronic acid in the majority of cases.

Fragments of sulfate dermatan, responding to the formula (B) herein above, wherein n can vary from 1 to 20, can be obtained by periodical oxidation followed by a reduction in the sodium borohydride and an acid hydrolysis, as

described in FRANSSON LA. and CARLSTEDT I, Carboydr. Res., 36 (1974) 349-358.

Glycosaminoglycans have numerous biological activities, among which can be cited their activities with regard to coagulation factors, that can be exercised by means of various plasmatic proteins. With regard to heparin, it is also known in the literature that heparin or certain of its derivatives may or may not have an anticoagulation activity that can have a regulatory activity in the proliferation of smooth muscular cells of the vascular wall (GUYTON et al., Circ. Res. 46 (1980, 625-634) or else an inhibiting activity of the heparan, enzyme involved in the metastatic dissemination mechanisms (request EP No. 254 067). In addition, glycosaminoglycans are part of the largest family of sulfated polysaccharides, certain having shown at more or less significant degrees an anti-viral activity (BABA et al., Antimicrob Agents Chemother, 32 (1988) 337-339) and in particular an anti-HIV activity (human-immunodeficiency virus) (BABA et al., Proc. Natl. Acad. Sci., 85 (1988) 6132-6136).

In the following part of the text, the GAG form will be used to designate a glycosaminoglycan which either has a natural structure such as obtained by extraction, semi-synthesis or synthesis, or a chemically modified structure on carboxyl or aminated functional clusters, prior to the acetylation reaction resulting in selectively O-acetylated GAGs.

In spite of their pharmacological significance, natural GAGs have the disadvantage of having a relatively short half-life, making it necessary for repeated administrations. This disadvantage has been in part offset by the use in the prevention and the treatment of venous thromboses, of heparin derivatives with a low molecular mass, administered subcutaneously, thus lowering the frequency of administration to one injection per day.

Nevertheless, there is an advantage in being able to have derivatives with a delayed action, which would make it possible to reduce the frequency of administration of these products still more by increasing the action time.

There can also be an advantage in having non-anti-coagulant delayed derivatives such as those of N-disulfate N-acetylated heparins whose activity as inhibitor of heparanasis, an enzyme involved in the phenomena of dissemination of metastasis and recalled above.

The literature describes a number of modified glycosaminoglycans derivatives so as to improve their pharmacokinetics, in particular esters of the carboxylic function of uronic acids or hydroxyl functions.

In particular the partial or total esterification of heparin carboxyl functions has been used, treated in the form of quaternary ammonium salt, in inert solvent, by an alcohol or a halogen derivative, in the presence potentially of a condensation agent (Br. FR No. N2 159 724 and EP no. 44 228). Hyaluronic acid derivatives obtained by esterification with the help of alcohol in the presence of a catalyst or by reaction with a etherifying agent have also been described (Br. EP no. 216 453).

Moreover, various means have been described in the literature in view of esterifying GAGs on primary and secondary hydroxyl functions.

The publication Can. J. Res., 25B (1947), 472-476, describes a heparin acetylated derivative corresponding to an acetyl group mole for four saccharide units. The product is prepared by ketone action on the heparin in acetone. Though in this publication, it is specified that the product obtained is an O-acetylated derivative, it is well known that the ketene in presence of carboxylic groups gives anhydrides (cf. J. March, Advanced Organic Chemistry; Reactions, Mechanisms and Structure, J. Wiley and Sons, Editions 1985, pp. 685-687: "With ketones, carboxylic acids give anhydrides and acetic anhydride is prepared industrially in this manner:

[formula]

Accordingly, this publication describes in fact a heparin in which O-acetyl groups are associated with mixed anhydrides between the COO^- group of the uronic acid and the acetyl group from ketene, which can explain their instability in water.

The patent FR 2 100 735 describes partial hydrolysable heparin esters and an non-toxic organic acid, specifically the 4-chlorophenoxyisobutyric acid, 4-chlorophenoxyacetic acid, cholaic acid, nicotinic acid, pyridylacetic acid, N-oxy-pyridylacetic acid or linolic acid. The preparation method described in patent above, that is characterized by the reaction of a quaternary heparin salt with the acid activated by a carbodiimide, gives not only the O-acetylated derivative, but also in a significant quantity a secondary stable product in the form of an O-acetylsulfated ester derivative. In addition, such a type of reaction is likely to promote the obtaining of anhydrides and intramolecular reactions between the acid functions and hydroxyl functions of the heparin.

Patent JP 74/048533 describes O-esters of sulfate chondroitin with aromatic acids, arylaphatic or heterocyclic acids possibly substituted with prolonged activity. The preparation method described in this document that is characterized by the reaction of the chondroitinsulfuric acid with an acid chloride gives an N-acetylated as secondary reaction.

Patent WO 83/00150 generally describes drug procedures prepared by using various functions of GAGs, whose hydroxyl and specifically an O-ester of the chondroitinsulfate with penicillin V. The preparation of this product is implemented by means of carbodiimide and has therefore secondary reactions mentioned herein above.

Patent EP 46 828 describes heparin O-esters with non-saturated acids, specifically the acrylic or metacrylic acid that, grafted onto biomedical materials, confer upon them a long-lasting antithrombin activity. This grafting is

implemented by covalent bonding at the level of non-saturated α- β bonds of these esters with the surface of said materials entering into contact with blood. According to this document, the non-saturated alpha, beta O-esters are prepared by reaction of the heparin with chloride or anhydride of an α carboxylic acid, β-non-saturated. Moreover, the description indicated indifferently the use of chlorides or non-saturated α, acid anhydrides as reactants do not specify by which types of O-esters of heparin are thus obtained.

Patent EP 256 880 describes the esterification of low-molecular weight heparin by the action of chloride acid in the formamide and the pyridine in order to give derivatives with increased transmembrane permeability. However, this method leads to derivatives that undergo a partial desulfication and are O- but also N-acetyls, and in which the sulfate/carboxyl relationship is altered.

Patent PR 3 066M describes the acetylation of the N-monomethylheparinamide by the action of the acetic anhydride in the formamide and the pyridine. The initial product is a derivative of heparin whose carboxyl functions are replaced by amide functions.

The process used for the acetylation does not use a soluble salt in organic environment. As a result, the reaction of acetylation cannot be precisely controlled and only makes it possible to obtain a low yield of acetylation. Moreover, if the process described with heparin is applied whose carboxyl functions are not blocked, a significant formation of anhydrides is obtained mixed between the heparin carboxyl groups and the acetic anhydride, these undesirable secondary products are not eliminated from the environment.

Patent JP 5128602 describes the acetylation of the heparin by the action of an acid anhydride in the formamide. In the process used, the heparin is not in the form of a soluble salt in an organic environment, which does not allow the precise controlling of the acetylation reaction and involves obtaining a low rate of acetylation. Moreover, the process described involves the presence in the mixed anhydride environment of secondary products from the reaction.

It has now been found that by making a soluble salt react in a GAG's organic environment, such as a polar aprotic salt, a selective acylation is obtained from free hydroxyls, without altering the carboxylic or aminated functional groups of the GAG used. The implementation of the GAG in the form of a soluble salt in organic environment advantageously allows the controlling of the acylation reaction with a significant alteration over the rest of the molecule. In particular, an acylation rate from 0.1 to 3 acyl groups is obtained per disaccharide unit, and in particular from 0.5 to 2 acyl groups. It has also been found that no N-acylated derivative is formed and that when anhydrides are formed at the level of carboxylic functions, the use of a weak base easily makes it possible to retransform into free acid.

Advantageously, the selective acylation of the GAGs according to the process of the invention allows a modulation of their biological activities, that in certain cases, is significantly increased.

Lastly, it has been found that the O-acylated GAGs thus obtained have a longer lasting pharmacological activity.

Thus the object of this invention, according to one of its aspects, of the selectively O-acylated glycosaminoglycans, respond to the following formula I:

(I)

wherein:
- G represents a group (a) of the formula:

(a)

or a group (a') of formula:

(a')

or a group (b) of formula:

(b)

_ U represents a group (c) of formula:

(c)

or a group (d) of formula:

(d)

or the residue of the group (c) or the group (d) after periodical oxidation followed by a β-elimination or acid hydrolysis:
- A represents a group R_1, a group R_1-(c), or a group R_1-(d), or a group (e) of formula:

(e)

or the residue of the group (c) or the group (d) after periodical oxidation followed by a B-elimination or an acid hydrolysis:

- B represents an O- R_1 group, an (a)-OR_1 group, an (a')- OR_1 group, a (b)- OR_1 group, a (f) group of the formula:

(f)

or a group (g) of the formula:

(g)

or represents a group (a), a group (a') or a group (b) to which remains connected a residue of (c) or (d) such as presents after periodical oxidation followed by a B-elimination or an acid hydrolysis:
- R_1 represents H_1SO_3 or acetyl, acetyl being the residue of a carboxylic acid or dicarboxylic non a-b non-saturated chosen from among:
- an alcanoyl group from 1 to 18 carbon atoms;
- an alcanoyl group from 2 to 3 carbon atoms replaced by:
- an cycloalkyl group from 3 to 7 carbon atoms;
- a phenyl group possibly replaced by one or more alkyl radicals from 1 to 14 carbon atoms, halogen atoms or NO_2 or OCH_3;
- a hydrcarbonated radical aliphatic non-saturated from 4 to 16 carbon atoms;
- a benzoyl group possibly replaced by one or more alkyl radicals from 1 to 4 carbon atoms, halogen atoms or NO_2 or OCH_3 atoms;
- a cyoloalkyl group (3-7 C) carbonyl;
- R_2 represents SO_3 and/or an acetyl radical, on condition that the proportion of N-acetyl glucosamine is at maximum equal to that of heparin when R_1 represents an acetyl radical;
- R_3 presents an atom of hydrogen or an alkyl radical with 1 to 10 carbon atoms, preferably from 1 to 4 carbon atoms, or a phenyl-alkyl radical with 7 to 12 carbon atoms, or a metal cation alkyl or alkaline-terra;
- n is a whole number from 1 to 80,
R_1 is acyl in a proportion of at lest o.1 to 3 acyl groups per disaccharide unit, preferably from 0.5 to 2 acyl groups, and their salts pharmaceutically acceptable.

In a preferred process of the invention, the glycosaminoglycans chosen are heparin-type glycosaminoglycans, i.e., the heparin the derivatives of heparin, obtained either by splitting, either by semi-synthesis or synthesis, sulfate heparan and its derivatives obtained by splitting, semi-synthesis or synthesis.

Glycosaminoglycans of this type, selectively O-acylated according to this invention, respond to the following formula II:

(II)

wherein:
- A has the meanings given above in formula (I):
R_1 represents H and/or SO_3- and/or an acyl group as defined in formula (I);
- R_2 represents SO_3- and/or an acetyl radical provided that the proportion of N-acetyl glucosamine is at most equal to that of heparin when R_1 represents an acetyl radical;
- R_3 represents an atom of hydrogen, an alkyl radial from 1 to 10 carbon atoms, preferably from 1 to 4 carbon atoms, an alkyl radical replaced by 7 to 12 carbon atoms, or a metal alkaline cation or alkaline-terra;
- B represents (a) – OR_1 or (f), (a) and (f) being such as defined in formula (I), or OR_1, or a group (a), to which a residue of (c) or (d) is hooked such as present after periodical oxidation followed by a β-elimination or an acid hydrolysis;
- n is a whole number from 1 to 80.

A preferred family IIa of compounds of the invention are compounds of formula II:

(II)

wherein – A has the meanings given above in formula (I)
- A has the meanings given above in formula (I):
R_1 represents H and/or SO_3- and/or an acetyl group as defined in formula (I);
- R_2 represents SO_3- and/or an acetyl radical provided that the proportion of N-acetyl glucosamine is at most equal to that of heparin when $_{R1}$ represents an acetyl radical;
- R_3 represents an atom of hydrogen, an alkyl radial from 1 to 10 carbon atoms, preferably from 1 to 4 carbon atoms, an alkyl radical replaced by 7 to 12 carbon atoms, or a metal alkaline cation or alkaline-terra;
- B represents (a) – OR_1 or (f), (a) and (f) being such as defined in formula (I), or OR_1, or a group (a), to which a residue of (c) or (d) such as present after periodical oxidation followed by a B-elimination or an acidhydrolysis
- n is a whole number from 1 to 80, provided that when A represents R_1, a group R_1-(c) or a group R_1-(d) and that B represents O-R_1, n is a whole number from 1 to 16.

Another preferred family IIb of compounds of the invention corresponds to compounds of formula II:

(II)

wherein
- A has the meaning given above in formula I;
- R_1 represents H and/or SO_3 and/or an acetyl group, acetyl being the residue of a carboxylic acid or dicarboxylic acid not a-b non-saturated cholei among:
- an alcanoyl group from 4 to 18 carbon atoms,
- an alcanoyl group from 2 to 3 carbon atoms, substituted by
- an cycloalkyl group from 3 to 7 carbon atoms,
a phenyl group possibly substituted by one or more alkyl radicals from 1 to 14 carbon atoms, halogen atoms or NO_2 or OCH_3 groups,
- an non-saturated aliphatic hydrocarbonated radical with 4 to 16 ca,
- benzoyl group possibly substituted by one or more alkylated radicals from 1 to 4 carbon atoms, halogen atoms or NO_2 or OCH_3 clusters;
- a cycloalkyl (3-7C) carbonyl cluster;

- R_2 represents SO3 and/or an acetyl radical subject to the proportion of N-acetyl glucosamine is a maximum equal to that of the heparine when R1 represents an acetyl radial;
- R_3 represents a atom of hydrogen or an alkyl radial from 10 to 10 carbon atoms, preferably from 1 to 4 carbon atoms, or a phenyl-alkyl radical with 7 to 12 carbon atoms, or an alkalin or alkalin-terrous metal cation;

Advantageous compounds of the invention are compounds belonging to families (IIa) and (IIb) in which R3 represents an atom of hydrogen or an alkalin or alkalin-terrous metal cation.

Other groups of advantageous compounds corresponding to compounds belonging to families (Iia) and (Iib), in which F3 represents an alkyl radical with 1 to 10 carbon atoms, preferably from 1 to 4 carbon atoms, or an alkyl radical substituted from 7 to 12 carbon atoms.

In a preferred aspect of the invention, mixtures of heparin fragments are used with a molecular mass less than 10,000 daltons, in particular either those with an average molecular mass between 2,000 and 7,000 daltons, or those with an average molecular mass of around 4,500 daltons, or yet those with an average molecular mass of around 2,500 daltons.

It is possible to advantageously use to obtain these a depolymerization process with nitrous acid as described for example in patent EP 37319. Selectively O-acylated glycosaminoglycans of the invention are then represented by the following formula III:

[formula III]

wherein:
- A represents R1 or F1-(c) or R1-(d) as defined in formula (I):
- R_1 represents H_1 and/or SO_3 and/or an acyl group as defined in formula (I);
- R_2 represents $-SO_3$ and/or an acetyl radical provided that the proportion of N-acetyl glucosamine is at most equal to that of heparin when R_1 represents an aceltyl radical

- R_3 represents a hydrogen atom, and/or an alkyl radical with 1 to 10 carbon atoms, preferably from 1 to 4 carbon atoms, or a phenyl alkyl radical with 7 to 12 carbon atoms, or an alkalin or alkalin-terrous metal cation;
- n is a whole number from 3 to 12

Preferred compounds of the invention correspond to compounds of formula (III) wherein R1 is an alkanoyl radical with 4 to 10 carbon atoms;

To obtain mixtures of heparin fragments with a molecular mass less than 10,000 daltons, it is possible to use an enzymatic depolymerization method, for example as described in patents EP 244235 and 244236 or alkalin depolymerization, for example as described in patent EP 40144.

In this case, selectively O-acylated glycosaminoglycans according to the invention are represented by the following formula IV:

[formula IV]

wherein:
- R_1 represents H_1 and/or SO_3 and/or an acyl group as defined in formula (I);
- R_2 represents SO_3- and/or an acetyl radical provided that the proportion of N-acetyl glucosamine is at most equal to that of heparin when R_1 represents an acetyl radical
- R_3 represents a hydrogen atom, and/or an alkyl radical with 1 to 10 carbon atoms, preferably from 1 to 4 carbon atoms, or a phenyl alkyl radical with 7 to 12 carbon atoms, or an alkalin or alkalin-terrous metal cation;
- B represents (a) $-OR_1$ as defined in formula (I) or DR_1;
- n is a whole number from 2 to 20

Among compounds of formula (IV) advantageous compounds are those in which R_1 is an alkanoyl radical with r to 120 carbon atoms.

In another advantageous aspect of the invention, a mixture of homogenous heparin fragments can be used with regard to their molecular mass, a fragment of heparin obtained by synthesis, homogeneous with regard to its molecular mass and with regard to its functionalization.

In another interesting aspect of the invention, glycosaminoglycans can be chosen from heparin derivatives devoided of bonding site at the antithrombine III (AT III) either that the heparin chains have been split so as to eliminate the oligosaccharidic chains comprising the bonding site at the AT III by resorting for example to a chromatography of affinity on the Sepharose-AT III resin or to ion exchange chromatrophs, as described in E. Sache et al., Thromb. Res. 25 (1982) pp. 442-458, or that these sites have been destroyed for example by periodical depolymerization followed by a β-elimination or an acid hydrolysis.

These compounds can respond to the following formula V:

[formula V]

wherein:
- A represents R_1–(c), R_1–(d) or the residue of (c) or (d) after periodical oxidation followed by a β-elimination or an acid hydrolysis;
- B represents (a)-OR_1, or a group (a) to which a residue of (c) or (d) remains attached as present after periodical oxidation followed by a β-elimination or an acid hydrolysis;
- R_1 represents the meanings given in formula (I);
- R_2 represents $-SO_3$ the proportion of $-SO_3$ or $-SO_4$;
- R_3 represents a hydrogen atom, and/or an alkyl radical or an alkalin or alkalin-terrous metal cation;
- n is a whole number from 1 to 80

Particularly advantageous compounds can be obtained starting from compounds prepared by the process implementing a periodical oxidation followed by alkalin β-elimination, a reduction and a splitting as described above. These compounds respond to the following formula VI:

[formula VI]

wherein:
- A represents R_1, R_1–(c), R_1–(d) or the residue of (c) or (d) after periodical oxidation followed by a β-elimination or an acid hydrolysis;

- U represents

[formula]

or due to a pattern for at least two chains, an non-sulfated uronic acid (D-glucuronic or L-iduronic) open between the carbons in positions 2 and e of the formula:

[formula]

- B represents (a) -OR_1, or OR_1, or a group (a) to which a residue of (c) or (d) remains attached as present after periodical oxidation followed by a β-elimination;
- R_1 represents the meanings given in formula (I);
- R_2 represents $-SO_3$ or an acetyl radical, the proportion of SO_3 being around 90%;
- R_3 represents a hydrogen atom, and/or an alkyl radical or an alkalin or alkalin-terrous metal cation;
- n is a whole number from 2 to 18.

Advantageous compound responding to formula VI are those in which n is a whole number from 7 to 15 for the majority of cases constituting them. Advantageous compounds responding to formulas V and VI, and in particular those responding to formula VI in which n is a whole number from 7 to 15 for the majority of cases, are those in which R1 is an alkanoyl radical with 2 to 10 carbon atoms, advantageously from 4 to 10, preferably r or 6 carbon atoms.

Other preferred compounds responding to formula VI are mixtures of homogenous compounds with regard to their molecular mass, obtained by gel-filtration, in which n is a whole number from 2 to 12.

Among the heparin structure compounds devoid of bonding site at AT III, and therefore devoid of anticoagulating activity, a family of advantageous compounds corresponds to N-desulfated N-acetylated heparin derivatives selectively O-acylated responding to the following formula VII:

[formula VII]

wherein:
- A represents R_1, R_1–(c), R_1–(d) or the residue of (c) or (d) after periodical oxidation followed by a β-elimination or an acid hydrolysis;
- R_1 represents the an alkanoyl radical with 2 to 18 carbon atoms
- R_3 represents a hydrogen atom, and/or an alkyl radical or an alkalin or alkalin-terrous metal cation;
- B represents (a) -OR_1, as defined in the formula (I) or OR_1;
- n is a whole number from 1 to 18.

In another advantageous aspect of the invention, glycosaminoglycans that are chosen are of sulfate-chondroitine-type, i.e., 4- and 6-sulfated chondroitines, sulfated dermatane and their fragments.

Glycosaminoglycans of this type, selectively O-acylated according to the invention are represented by the following formula VIII:

[formula VIII]

wherein:
- A has the meanings of formula (I);
- R_1 represents H and/or SO_3- and/or an acyl group as defined in formula (I);
- R_3 represents a hydrogen atom, an alkyl radical with 1 to 10 carbon atoms, preferably from 1 to 4 carbon atoms, or a phenyl-alkyd radical with 7 to 12 carbon atoms, or an alkalin or alkalin-terrous metal cation;

- B represents (b) -OR_1, or (g) as defined in formula (I) or OR_1 or a group (b) to which a residue of (c) or (d) remains attached as present after periodical oxidation followed by a β-elimination or an acid hydrolysis;
- n is a whole number from 1 to 80.

Among the compounds of formula (VIII), a family advantageously corresponds to the compounds wherein R_3 represents a hydrogen atom or an alkalin or alkalin-terrous metal cation.

Other advantageous compounds of formula (VIII) are those in which R3 represents an alkyl radical with 1 to 10 carbon atoms, preferably from 1 to 4 carbon atoms, or a phenyl-alkyl radical from 7 to 12 carbon atoms.

Another object of the invention is a preparation process of selectively O-acylated glycosaminoglycans.

The process of this invention is characterized by that the glycosaminoglycan is transformed into a soluble salt in organic environment and in that this salt is treated by an acylation agent capable of acylating selectively the primary and secondary hydroxyl clusters of this glycosaminoglycanes, without altering the NHR_2 or $COOR_3$ functional clusters present on this glycosaminoglycanes before the acylation reaction, the reaction taking place in a polar aprotic solvent in the present of a catalyst and in the presence of a base capable of capturing the acid released during acylation, at a temperature from 0°C to 100°C.

[formula]

wherein : -G° represents a group (a)° of the formula:

[formula a]

or a group (a') of formula:

[formula]

or a group (b)° of formula:

[formula]

- U represents a group (c)° of formula:

[formula]

or a group (d)° of formula:

[formula]

or the residue of group (c)° or group (d)° following periodic oxidation followed by a β-elimination or an acid hydrolysis;
-A° represents a group R_1°, a group R_1°-(c)°, a group R_1°-(d)°, or a group (e)° with the formula:

(e)°

or the residue of group (c)° or group (d)° following periodic oxidation followed by a β-elimination or an acid hydrolysis;

- B° represents a group OR_1°, a group (a)°-OR_1°, a group (a')°-OR_1°, a group (b)°-OR_1°, or a group (f)° having the formula:

(f)°

Or a group (g)° having the formula:

or represents the groups (a)°, (a')°, or (b)° to which a residue of (c)° or (d)° remains attached as present after periodical oxidation followed by a β-elimination or an acid hydrolysis.

- R_1 represents H or SO3;
- R_2 represents SO3 or an acetyl provided that the proportion of N-acetyl glycosamine is at the most equal to that of the heparin when R_1 represents an acetyl radical;
- R_3 represents a hydrogen atom, and/or an alkyl radical with 1 to 10 carbon atoms or a phenyl-alkyl radical with 7 to 12 carbon atoms or an alkalin or alkalin-terrous metal cation;
- n is a whole number from 1 to 80

in a salt of said soluble glycosaminoglycane in a polar aprotic organic solvent;
(2) this salt is treated by an anhydride of the formula:
Acyl – O – Acyl

in which Acyl is as defined in formula (I) in said polar aprotic organic solvent, in the presence of catalytic quantities of pyridine or a dialkylaminopyridine and a proton acceptor;
(3) the product thus obtain by action of a sodium acetate solution in the ethanol is precipated; and
(4) the selectively O-acylated glycosaminoglycan is isolated by dissolution in the water of the precipitate thus obtained and by dialysis in the presence of a weak base and the sodium salt of the selectively O-acylated glycosaminoglycan that is obtained is possibly transformed into another pharmaceutically acceptable salt.

In an advantageous aspect of the invention's process, the glycosaminoglycan used in phase (1) is chosen in the group constituted by the heparin, a mixture of heparin fragments with a molecular mass less than 10,000 daltons, a heparin fragment mixture with an average molecular mass between 2,0000 and 7,000 daltons, a heparin fragment mixture with an average molecular mass of around 4,500 daltons, a heparin fragment mixture with an average molecular mass of around 2,500 daltons, a heparin fragment mixture homogenous with regard to their molecular mass, a heparin fragment obtained by synthesis, homogeneous with regard to its molecular mass with regard to its functionalization.

In another advantageous aspect of the invention's process, the glycosaminoglycan used in phase (1) is heparin, a fraction of a heparin fragment devoid of bonding site to the antithrombin III.

The glycosaminoglycan used in phase (1) of the invention's process may still advantageously be chosen in the group constituted by the dermatan sulfate and its fragments, or the chondroitins 4- and 5-sulfate and their fragments.

Advantageously, the glycosaminoglycans salt used in phase (1) of the invention's process is a tertiary amine salt, in particular, a tributylammonium salt , or a quaternary ammonium salt, in particular a tetrabutylammonium salt.

In another advantageous aspect of the process of the invention, the anhydride used in phase (2) is the anhydride of an alcanoic acid containing from 2 to 10 carbon atoms advantageously from 4 to 10, preferably 4 or 6 carbon atoms.

The polar aprotic solvent wherein phase (2) of the process of the invention is conducted can advantageously be chosen from among the group constituted by dimethylformamide, hexamethylphosphorotriamine, pyridine, or else a mixture of these solvents among themselves or with dichloromethane and the catalysis among the group constituted by amines such as pyridine and dimethylaminnnopyridine.

The base intended to neutralize the acidity can be pyridine (that can be used both as solvent, catalyst and base) triethylamine or tributylamine.

Advantageously, the dialysis performed in phase (4) is done in the presence of a weak based such as sodium bicarbonate in order to eliminate any secondary products such as anhydrides.

Advantageously the temperature and the duration of the reaction can vary, according to the desired acetylation rate, respectively from 0°C to 100°C, in

particular from 0°C to 40°C and advantageously at ambient temperature for 1 to 24 hours, for example.

The compounds of the invention, selectively O-acetylated, in vivo a long-lasting activity. For example, when these compounds are heparin-type compounds with the bonding site at AT III, and are therefore susceptible of exercising an antithrombin activity, this activity is clearly prolonged over time with respect to the same compound not having undergone a selective O-acetylation.

The selectively O-acetylated heparin derivatives according to the invention whether or not they have the bonding site at AT III, the latter are practically devoid of anticoagulating activity with also various biological activities, in particular:

- the inhibition of proliferation of smooth muscular cells, that have an advantage to avoid restenosis in the interventions such as angioplasty, potages and venous grafts and arterial, organ transplants, in particular heart transplants.
- the inhibition of heparan and heparin, enzymes involved in the metastatic dissemination, anti-viral properties, in particular with regard to retrovirus, in particular with regard to various HIV (human-immunodeficiency virus) which have an interest in the treatment of AIDS,
- properties for inhibiting the leucocyte elastase, increase in the rate of circulants of elastase inhibitors and the selective inhibition of the type-III collagen synthesis and fibroactine, which provides them advantages in the treatment of diseases in which an imbalance of the elastase-anti-elastase system is involved, such as emphysema, and the treatment of degenerative diseases of the conjunctive tissue such as arteriosclerosis and diabetes.

The selectively O-acetylated glycosaminoglycans of the invention can therefore constitute the principle ingredient of very important drugs in a number of indications.

Another object of the invention are pharmaceutical compounds characterized in that they enclose as an active substance quantity efficient of at least a O-acetylated glycosaminoglycan according to the invention, in association with a pharmaceutically acceptable vehicle.

Advantageously, the selectively O-acetylated glycosaminoglycans are in the form of a pharmaceutically acceptable salt, such as a magnesium or calcium sodium salt.

In an advantageous implementation of the invention, the pharmaceutical compounds are characterized in that the pharmaceutical vehicle is appropriate for oral administration and that they are present in the form of drinkable solutions, advantageously enclosed from 50 mg to 5 g per unit, preferably from 100 to 1000 mg for capsules, tablets or pills and from 10 to 150 mg for drinkable solutions, for example one to three times per day; or else in that these compounds are present in the form of injectable solution, sterile or that can be sterilized, for the intravenous administration, intramuscular or subcutaneous administration, these solutions advantageously enclose from 50 to 200 mg/ml of O-acetylated glycosaminoglycan when they are intended for injection subcutaneously or from 20 to 200 mg/ml of selectively O-acetylated glycosaminoglycans when they are intended for injection intravenously or by perfusion.

These intervals of doses only have an indicative value, the doses administered must, in each case, be evaluated by the clinician, considering the status of the patient and its personal activity with regard to drugs.

The invention also concerns by way of biological reaction, the O-acetylated glycosaminoglycans of the invention that can be used as references or markers in comparative studies for studies of structure/activity relationships in various physiological systems in which the glycosaminoglycan can be involved.

The invention will better be understood by means of the applicative examples following that are in no way limitative.

EXAMPLE 1: Preparation of O-acetylated heparin (IC 1938) based on tetrabutylammonium salt of heparin

a) Preparation of the heparin tetrabutylammonium salt:

the heparin sodium salt (10 g) dissolved in water (500 ml) is percolated through a column of resin exchange of cations (Dowex 50 W x 4, in the form of H+). The solution obtained is neutralized by tetrabutylammonium hydroxide. After lyophilization the heparin tetrabutylammonium salt is obtained (19.55 g).

b) Acetylation:

1.05 g of heparin of tetrabutylammonium is dissolved in dimethylformamide anhydrous (4 ml). After cooling to 0°C, acetic anhydride is added drop-by-drop (1 ml: 10.46 mmoles) then triethylamine (1.45 ml: 10.46 mmoles) and dimethylaminopyridine (64 mg: 0.5 mmoles). the mixture is abandoned 24 hours at ambient temperature. After adding water (4 ml), the solution is dialyzed for 72 hours against distilled water. The tetrabutylammonium salt is converted into sodium salt by passing over a Dowex resin 50, H+ at 0°C, followed by neutralization by 1N sodium. After lyophilization, 0.49 g of heparin of sodium is obtain selectively O-acetylated with the following characteristics:

Relationship sulfate/carboxyl:	2.28 meq/g initial product: 2,20 meq/g)
APTT titre:	91 ul/mg

Spectrum ^{13}C RMN (methanol 51.5 ppm, standard internal)
- signal at 23.4 ppm: CH_3 from CH_3-CO-O.
- signal at 24.5 ppm (weak: CH_3 of CH_3-CO-NH-
(identical to the initial product)

The RMN spectrum indicates the presence of around two acetyl groups by disaccharide unit.

EXAMPLE 2: Preparation of O-acetylated heparin (IC1938) based on heparin tributylammonium salt.

a) Preparation of the heparin tributylammonium salt:

The heparin sodium salt (10 g) is dissolved in water (500 mo) then percolated through a column of exchanging resin of cations (Dowex 50 W x 4, in

H+ form). The solution is neutralized by adding a solution of tributylamine at 10 % in ethanol. After watering with ether and lyophilization, the heparin tributylammonium salt is obtained (14.77 g).

b) Acetylation:

To a solution cooled to O° C of the above salt (4 g) in anhydrous dimethylformamide (50 ml) is added dimethylaminopyridine (250 mg), acetic anhydride, (3.9 ml) and tributylamine (9.7 ml). After 24 hours at room temperature, water (1.5 ml) is added. The mixture is then poured into a solution of saturated ethanol in sodium acetate. After washing with ethanol, the precipitate is dissolved in water, then dialyzed against bicarbonate at 5% in water, then against water. After lyophilization, o-acetyl sodium heparinate is obtained (1.81 g).

The infrared spectrum presents a strong ester band at 1730 cm^{-1}.

After saponification, the product presents:

- a sulfate/carboxyl ration of 2.38 meq/g (initial product: 2.40 meq/g).
- an APTT titer of: 85 ul/mg.

Example 3: Preparation of o-acetylated heparine (IC 1938) using pyridine catalyse

Heparine tetrabutylammonium salt (0.58 g) is dissolved in a mixture (1/1; v/v) of pyridine and dimethylformamide (10 ml). Acetic anhydride is added (0.5 ml), then the mixture is left at room temperature. After 24 hours, one adds an aqueous solution of sodium acetate (1 M, 15 ml) then the mixture is poured into ethanol (80 ml) cooled to O°. After centrifuging, the precipitate is redissolved in water (10 ml), then ethanol (160 ml) is added, followed by aqueous sodium acetate (1 M, 10 ml). The precipitate is then retaken by water and lyophilized, yielding o-acetylated heparin (0.22 g).

EXAMPLE 4: preparation of o-propionylated heparin (IC 1939) with different degrees

To a cooled solution at 0°C of a solution of tetrabutylammonium heparinate (1.85 g), prepared as described in Example 1, in dimethylformamide (10 ml) propionic anhydride is dripped (2.7 ml) and then triethylamine (2,9 ml) and dimethylaminopyridine (128 mg). After 1 h, 2 h, 4 h, 8 h, and 24 h, a part of the of the reaction mixture is taken, diluted with an equal volume of water and dialyzed for 24 hours in distilled water. After passing through Dowex 50 H^+ resin and neutralizing with caustic soda, the resulting products are lyophilized and have the following characteristics:

Reaction time	Mass	Sulfate/Carboxyl meo/g	APTT (ui/mg)
24 h	496 mg	2,31	80
8 h	138 mg	2,35	88
4 h	122 mg	2,33	94
2 h	120 mg	2,42	109
1 h	120 mg	2,33	120
(starting product)		2,40	150

The NMR proton spectrum of the products is recorded in water with 3,3,3- trimethylsilylpropionate (TSP) as internal standard. It has signals at ~ 1.1 ppm and ~ 2.4 ppm, characteristic of the radicals CH_3 and CH_2 of CH_3-CH_2-CO-O, respectively, and signals between ~ 3 and ~ 5 ppm, characteristic of the protons of the osteal skeleton.

The comparison of intensity of the signals of propionyl and skeleton in the product treated for 1 hour with the product treated for 24 hours as per the above reaction shows the effect of the reaction time : in fact, the signals of the skeleton protons decrease, which indicates to an increase in the degree of substitution.

The carbon spectrum (methanol 51.6 ppm, i. stnd.): signals at ~ 10.8 and ~ 30 ppm, characteristic of CH_3 and CH_2 radicals of propionate esters.

EXAMPLE 5 : preparation of o-butyrylated heparin (IC 1940)

A) Use of tetrabutylammonium salt of heparin:

To a solution of tetrabutylammonium salt of heparin (0.55 g) obtained as described in Example 1 in dimethylformamide (5 ml) is added at 0°C butyric anhydride and dimethylaminopyridine. After 24 hours, at room temperature, water (5 ml) is added, and the reaction mixture is then dialyzed for 72 hours with distilled water. After exchange on Dowex 50 H^+ and neutralizing with caustic soda and lyophilization, O-butyrylated heparin is obtained in the form of sodium salt (0.32 g).

Rapport sulfate/carboxyl : 2,15 meq/g

Starting product : 2,20 meq/g)

Activity APTT : 59 ui/mg

The NMR proton spectrum (TSP, i. std.) shows the presence of signals at 0.9 ppm, 1.6 ppm, and 2.4 ppm, characteristic of the groups CH_3-CH_2 de CH_3-CH_2-CH_2-CO-O- and signals between 3 and 6 ppm, characteristic of the osteal skeleton.

The analysis of the NMR spectrum shows the presence of about one butyryl chain per one disaccharide unit.

B) Use of tributylammonium salt of heparin:

To a solution of tributylamine heparinate cooled to 0°C (4 g), obtained as described in Example 2, in dimethylformamide (50 ml) is added dimethylaminopyridine (0,25 g), butyric anhydride (6.7 ml), and tributylamine (9.7 ml). The reaction mixture is allowed to stay at room temperature for 24 hours. Then water is added (1.5 ml) and then, after 30 minutes, a saturated solution of sodium acetate in ethanol (250 ml). The precipitate is then washed three times with ethanol and then dialyzed with A 5% solution of bicarbonate and then with water. After lyophilization, sodium salt of O-butyrylated heparin is obtained (2.1 g).
Activity APTT : 29 ui/mg

After saponification of esters with 0.6 M caustic soda for 2 hours at 0°C the resulting product has a sulfate/carboxyl ratio of 2.36 meq/g (2.40 meq/g in the starting product).

EXAMPLE 6 : Preparation of O-hexanoylated heparin (IC 1941)

A) Use of totrabutylammonium salt of heparin:

To a solution of tetrabutylammonium salt of heparin (0.55 g), obtained as described in Example 1, in dimethylformamide (5 ml), are added at 0°C caproic anhydride (1 ml ; 6 mmoles), triethylamine (0.84 ml, 6 mmoles), and dimethylaminopyridine. After 24 hours, at room temperature, water (5 ml) is added, and the reaction mixture is then dialyzed for 72 hours with a 5% solution of bicarbonate and then with distilled water. After exchange on Dowex 50 H^+, neutralization with caustic soda, and lyophilization, O-hexanoylated heparin is

obtained as sodium salt (0.30 g).
Activity APTT : 25 ui/mg

The NMR proton spectrum (TSP, i. std.) has signals at 0.8, 1.2, 1.5, 2.3 ppm characteristic of CH_3-CH_2 of CH_3-$(CH_2)_8$-CO-O-

B) Use of tributylammonium of heparin:
To a solution of tributilammonium heparinate cooled to 0°C (4 g), obtained as described in Example 2, in dimethylformamide (50 ml) are added dimethylaminopyridine (0.25 g), tributylamine (9.7 ml), and hexanoic anhydride (10.6 ml). After 24 hours, at 20°C, water is added (1.5 ml) and then a saturated solution of sodium acetate in ethanol. After washing with ethanol, dialysis and lyophilization O-hexanoylated heparin was obtained (2.5 g).

EXAMPLE 7 : Preparation of O-octanoylated heparin (IC 1942)

To a solution of tributilammonium heparinate cooled to 0°C (4 g), obtained as described in Example 2, in dimethylformamide (50 ml) are added dimethylaminopyridine (0.25 g), octanoic anhydride (12.1 ml), and tributylamine (9.7 ml). After 24 hours, at room temperature, water is added (1.5 ml) and then, after 30 minutes, a saturated solution of sodium acetate in ethanol. After dialysis with a 20% solution of ethanol and then distilled water and ultrafiltration, the product is subjected to cation exchange on Dowex 50 H^+, with subsequent neutralization with caustic soda. After lyophilization, O-octanoylated heparin was obtained (2.5 g).

EXAMPLE 8 : PREPARATION OF O-DECANOYLATED HEPARIN (IC 1943)

A) USE OF TETRABUTYLAMMONIUM SALT OF HEPARIN:
To a solution of tetrabutylammonium salt of heparin (0.55 g),I obtained as described in Example 1, in dimethylformamide (5 ml), are added, at 0°C, capric anhydride (1 ml, 6 mmoles), triethylamine (0.84 ml, 6 mmoles), and dimethylaminopyridine. After 24 hours, at room temperature, water is added (5 ml), and the reaction mixture is then dialyzed for 72 hours with a 5% bicarbonate solution and then with distilled water. After exchange on Dowex 50 H^+, neutralization with caustic soda and lyophilization, O-decanoylated heparin is obtained as sodium salt (0.30 g).
The NMR proton spectrum (TSP, i. std.) has signals at 0.8, 1.2, 1.5, and 2.4 ppm characteristic of CH_3 and CH_2 of CH_3-$(CH_2)_8$-CO-O-

B) USE OF TRIBUTYLAMMONIUM SALT OF HEPARIN
To a solution of tributilammonium heparinate cooled to 0°C (4 g), obtained as described in Example 2, in dimethylformamide (50 ml) are added dimethylaminopyridine (0.25 g), decanoic anhydride (13.3 g dissolved in 20 ml of dimethylformamide ml), and tributylamine (9.7 ml). After 24 hours, at room temperature, water is added (1.5 ml) and then a saturated solution of sodium acetate in ethanol. The precipitate is dissolved in dimethylsulfoxyde and is then dialyzed with water, with sodium bicarbonate, and again with water.
After lyophilization, O-decanoylated heparin was obtained (2.38 g).

EXAMPLE 9 : PREPARATION O-OLEOLATE HEPARIN (IC 2013)

Tributylammonium salt of heparin (3 g) and N,N-dimethylaminopyridine (244 mg) are dissolved in anhydrous dimethylformamide(50 ml). After cooling to 0°C the following substances are dripped: oleic anhydride (21 g) synthesized as per Plusquellec et al, Tetrahedron, 1988, 44, 2471-2476, in a solution in dichloromethane (20 ml), then tributylamine (9.5 ml). After 24 hours of the reaction and cooling to 0°C, 5-% sodium bicarbonate was added (10ml) and then, after one hour, a saturated solution of sodium acetate. After washing with absolute ethanol, solubilization in a mixture of dimethylsulfoxyde and water (4/1, v/v, 750 ml), dialysis is conducted with a 10% aqueous solution of ethanol for 2 days and then with water for 3 days. Oleolate heparin salt is isolated after lyophilization and precipitation in of the lyophilizate, resolubilized in DMF (2,77 g). The content of oleic acid is: 1,44 µmole/mg (dosage as per Ducombe W. et al, Biochemical Journal, 1963, 88, 7).

EXAMPLE 10 : PREPARATION OF O-BENZOYLATED HEPARIN (IC 1944)
Tetrabutylammonium salt of heparin, obtained as described in Example 2, is benzoylated with benzoic anhydride under the conditions described above in the preparation of O-decanoylated heparin (Example 8).
The NMR carbon spectrum of the resulting product has signals at 131,132 and 136 ppm characteristic of benzoylated groups.
The product is stripped off the anticoagulation activity in vitro.

EXAMPLE 11 : PREPARATION OF O-3-CYCLOPENTYLPROPYONILATED HEPARIN (IC 2014)
3-cyclopentylpropionic anhydride is prepared using the procedure of Plusquellec et al, Tetrahedron, 1988, 44, 2471-2476. It is prepared by adding acid (23 ml, 150 mmoles) in a solution in dichloromethane (400 ml) to a stirred mixture cooled to -10°C containing tetrabutylammonium bromide (15 mmoles), caustic soda 20% (60 ml), and dichloromethane (80 ml), after decantation, washing with a 5-% sodium bicarbonate and water, and

concentration in oil (96%). The characteristic IR frequencies in IR: 1800, 1745, 1040 cm^{-1}.

Tributylammonium salt of heparin (4g) and N,N-dimethylaminopyridine (253 mg) are dissolved in dehydrated dimethylformamide (40 ml). After cooling to 0°C. the following substances are dripped: 3-cyclopentylpropionique anhydride (11g), then tributylamine (9,8 ml). After 24 hours of the reaction at room temperature and subsequent cooling to 0°C, water (1 ml) is added and then, in one hour,, a saturated solution of sodium acetate. After washing in absolute ethanol, the precipitate is dialyzed with a 5-% sodium bicarbonate for 24 hours, then with water for 3 hours. After lyophilization, O-3-cyclopentylpropyonilated heparin is obtained as sodium salt (2.64g).

The NMR carbon spectrum in D_2O (methanol at 51.6 ppm, i. stnd) has characteristic signals at 27,4 ppm; 33.1 ppm, 34.6 ppm, 35.9 ppm, and 41.7 ppm of O-cyclopentylproplonyl group. The sulfate/carboxyl ratio is 2.2.

EXAMPLE 12 : PREPARATION OF LOW-MOLECULAR O-ACETYLATED HEPARIN (MMW - 4,500 DALTONS, MW RANGE - 1,800-8,000 DALTONS) 0-ACETYLEE (IC 1945)

This low-molecular heparin is prepared by the partial nitrous depolymerization and alcoholic fractionation as described in patent EP 181252 and is referred to below as CY 216.

A) Use of tetrabutylammonium salt of CY 216

Sodium salt of CY 216 (1 g) is converted to tetrabutylammonium salt by passing through a column with Dowex 50 H^+ resin, with subsequent neutralization with tetrabutylammonium hydroxide.

The resulting salt (1.7 g) is dried in vacuum for three hours at 50°C and is then dissolved in anhydrous dimethylformamide (10 ml). After cooling to 0°C, acetic anhydride (1.7 ml) was dripped followed by triethylamine (2.4 ml) and dimethylaminopyridine (102 mg). After 20 hours of the reaction, the product is chromatographed in Sephadex Q-25 column and eluted with water. After the conversion in a sodium salt and lyophilization, O-acetylated CY 216 is obtained (0.89 g).

The NMR carbon spectrum (methanol 51.6 ppm, i. stnd.) has a signal at 23 ppm characteristic of acetates.

The signal of CH_3 of CH_3-CO-NH- at - 24.5 ppm is identical to that of the starting product.

The sulfate /carboxyl ratio is 2.09 meq/g (starting product: 2.05 meq/g)

- Activity APTT : 18 ui/mg
- Activity anti-Ax : 205 u/mg (Dosage as per Yin et al., J. Lab. Clin. Med., 1973, 81, 298-310)

B) Use of tributylammonium salt of CY 216

A sodium salt of CY 216 is converted to tributylammonium salt by passing through a column of Dowex 50 H^+ resin followed by neutralization with tributylamine as described for heparin. Tributylammonium salt of CY 216 is prepared by washing with ether, lyophillzation, and drying in drying cabinet in vacuum.

The above-described salt (4 g) and N,N-dimethylaminopyridine (288 mg) are dissolved in dimethylformamide. After cooling to 0° C, acetic anhydride (4.4 ml) is dripped followed by tributylamine (11.2 ml). After 24 hours at room temperature and after cooling to 0°C, water is added (1.7 ml) followed, in one hour, by a saturated alcoholic solution of sodium acetate. The precipitate is washed with ethanol, solubilized with apyrogenic water, dialyzed for 36 hours with a 5-% bicarbonate and then with water for 3 days. After lyophilization, acetylated CY 216 is obtained as sodium salt (1.4 g).

The NMR carbon spectrum in D_2O (methanol at 51.6 ppm as i. stnd.) has at 23 ppm a signal characteristic of acetylated group. The sulfate/carboxyl ratio is 2,07.

EXAMPLE 13: PREPARATION OF LOW-MOLECULAR O-BUTYRYLATED HEPARIN (MMW 4,500 DALTONS, MW RANGE - 1,800-8,000 DALTONS) (IC 1957)

Tributylammonium salt of CY 216 (4 g), obtained as described in Example 12, and N,N-dimethylaminopyridine (288 g) are dissolved in anhydrous dimethylformamide (40 ml). After cooling to 0°C, butyric anhydride (7.68 ml) is dripped followed by tributylamine (11.2 ml). After 24 hours of the reaction at room temperature and after cooling to 0°C, water is added (1.7 ml) followed, in one hour, by a saturated alcoholic solution of sodium acetate. After washing with ethanol, solubilization in apyrogenic water, dialysis with a 5-% sodium bicarbonate for 36 hours and then with water for 3 days, O-butyrylated CY 216 is isolated as sodium salt after lyophilization (2.14 g).

The NMR carbon spectrum in D_2O (methanol at 51.6 ppm, i. stnd.) has at 15.6 ppm, 20.5 ppm, and 39,4 ppm signals characteristic of O-butyryl group. The sulfate/carboxyl ratio is 2,08.

EXAMPLE 14: PREPARATION OF LOW-MOLECULAR O-HEXANOYLATED HEPARIN (MMW - 4,500 DALTONS, MW RANGE - 1,800-8.000 DALTONS) 0-HEXANOYLEE (IC 1958)

Tributylammonium salt of CY 216 (4 g), obtained as described in Example 12, and N,N-dimethylaminopyridine (288 g) are dissolved in anhydrous dimethylformamide (40 ml). After cooling to 0°C, capric anhydride (10.8 ml) is dripped followed by tributylamine (11.2 ml). After 24 hours of the reaction at room temperature and after cooling to 0°C, water is added (1.7 ml) followed, in one hour, by a saturated alcoholic solution of sodium acetate. After washing with ethanol, solubilization in apyrogenic water, dialysis with a 5-% sodium bicarbonate for 36 hours and then with water for 3 days, O-caproylated CY 216 is isolated as sodium salt after lyophilization (2.5 g).

The NMR carbon spectrum in D_2O (methanol at 51.6 ppm, i. stnd.) has at 15.9 ppm, 24.2 ppm, and 26,4 ppm, 33.1 ppm and 36.4 ppm signals characteristic of O-caproyl group. The sulfate/carboxyl ratio is 2,08.

EXAMPLE 15: PREPARATION OF LOW-MOLECULAR O-OCTANOYLATED HEPARIN (MMW - 4,500 DALTONS, MW RANGE - 1,800-8,000 DALTONS) (IC 1959)

Tributylammonium salt of CY 216 (4 g), obtained as described in Example 12, and N,N-dimethylaminopyridine (288 g) are dissolved in anhydrous dimethylformamide (40 ml). After cooling to 0°C, caprylic anhydride (14 ml) is dripped followed by tributylamine (11.2 ml). After 24 hours of the reaction at room temperature and after cooling to 0°C, water is added (1.7 ml) followed, in one hour, by a saturated alcoholic solution of sodium acetate. After washing with ethanol, solubilization in apyrogenic water, dialysis with a 5-% sodium bicarbonate for 36 hours and then with water for 3 days, O-octanoylated CY 216 is isolated as sodium salt after lyophilization (1.76 g).

The NMR carbon spectrum in d^6 DMSO (methanol at 51.6 ppm, i. stnd.) has at 16.8 ppm, 25.0 ppm, 27.3 ppm, 30.9 ppm, 31.4, 34.1, and 36,4 ppm signals characteristic of O-capryloyl group. The sulfate/carboxyl ratio is 2,07.

EXAMPLE 16: PREPARATION OF LOW-MOLECULAR O-DECANOYLATED HEPARIN (MMW - 4,500 DALTONS, MW RANGE - 1,800-8.000 DALTONS) (IC 1960)

Tributylammonium salt of CY 216 (4 g), obtained as described in Example 12, and N,N-dimethylaminopyridine (288 g) are dissolved in anhydrous dimethylformamide (40 ml). After cooling to 0°C, decanoic anhydride (15.3 ml) is dripped followed by dimethylformamide (10 ml) and then tributylamine (11.2 ml). After 24 hours of the reaction at room temperature and after cooling to 0°C, water is added (1.7 ml) followed, in one hour, by a saturated alcoholic solution of sodium acetate. After washing with ethanol, solubilization in apyrogenic water, dialysis with a 5-% sodium bicarbonate for 2 days, with 10-% NaCl for 2 days, and then with water for 5 days, O-decanoylated CY 216 is isolated as sodium salt after lyophilization (2.76 g).

The NMR carbon spectrum in D_2O (methanol at 51.6 ppm, i. stnd.) has at 16.4 ppm, 22.3 ppm, 25.1 ppm, 29.2 ppm, 31.9, 34.4, and 36.5 ppm signals characteristic of decanoyl group. The sulfate/carboxyl ratio is 2,13.

EXAMPLE 17 : PREPARATION OF LOW-MOLECULAR O-ACETYLATED HEPARIN (MMW - 2,500 DALTONS, MW RANGE - 1,500-8,000 DALTONS), (IC 1946)

This low-molecular heparin is obtained by the partial nitrous depolymerization using the procedure described in patent EP 37 319 and is referred to below as CY 222.

Sodium salt of CY 222 is converted to tributylammonium salt by passing through a column with Dowex 50 H^+ resin, with subsequent neutralization with tributylamine.

The resulting salt (1.5g) is dissolved in dimethylformamide (5ml). After cooling to 0°C, acetic anhydride (1.35 ml) is dripped followed by triethylamine (2 ml) and dimethylaminopyridine (85 mg). After 18 hours of the reaction, water is added (20 ml), and the mixture is dialyzed for 3 hours with distilled water. After conversion to sodium salt and lyophilization, O-acetylated CY 222 is obtained (0,86 g).

The product has the sulfate/carboxyl ratio of 1.98 meq/g (starting product: 1.97 meq/g).

The NMR carbon spectrum (methanol 51.6 ppm, i. stnd.) has a signal at 23 ppm characteristic of O-acetyl. The comparison of intensities of the signals of N-acetyl (at ~ 24.5 ppm) between the starting product and the resulting product shows that the acetylation is selective.

- Activity APTT : 8 ui/mg
- Activity anti-Xa : 191 u/mg (Dosage as per Yin et al., J. Lab. Clin. Med., 1973, 81, 298-310)

EXAMPLE 18 : A) PREPARATION OF HEPARIN FRAGMENT DEVOID OF AFFINITY TO ANTITHROMBIN III (IC 1772)

1/ Breaking the chains of heparin with a periodic acid :

10 g of injection heparin of pig mucus in the form of sodium salt 157 ui/mg strong in Codex dosage and 155 u/mg in anti-factor **Xa** dosage as per Yin et al. are dissolved in 250 ml of demineralized water at 4°C. pH of the solution is adjusted to 5.0 with concentrated hydrochloric acid. 10 g of sodium metaperiodate ($NaIO_4$, MW : 213.89) in a solution in 250 ml of demineralized water at 4°C are added under moderate stirring. pH of the mixture is adjusted 5.0 par with concentrated hydrochloric acid. The solution is allowed to stay for 24 hours in darkness in a cold chamber at +4°C.

2/ Removal of residual periodate:

The reaction solution is then distributed into three dialysis vessels NOJAX 40^R, (porosity of 3 to 4,000 Da) and was subjected to dialysis for 15 hours with running demineralized water.

3/ Depolymerizsation in a basic medium:

To 780 ml of the solution obtained after the dialysis are added 16 ml of 10N caustic soda, and the mixture was stirred for 3 hours at room temperature (about 18-21°C).

4/ Reduction:

500 mg of sodium borane $NaBH_4$, MW: 37.83) are then added, and the solution is again stirred for 4 hours at room temperature. pH of the solution is then adjusted to 4 with concentrated hydrochloric acid. After stirring for

15 minutes, pH is adjusted to 7 with concentrated caustic soda.

To 820 ml of the resulting solution are added 16.4 g of NaCl and 1270 ml of ethanol. The mixture is allowed to stay for 3 hours and is then centrifuged at 2,500 RPM for 20 minutes. The precipitate is collected, resuspended in 200 ml of ethanol, stirred in Ultra-Turrax® and finally recovered by filtration on fritted Buchner. The substance is then dried with suction at 40°C for 5 hours .

8.9 g of the product are obtained.

5/ Alcoholic fractionation:

The resulting 8.9 g of the product are dissolved in about 120 ml of demineralized water at room temperature. 1.78 g of NaCl are added, and pH of the solution is reduced to 3,5 with hydrochloric acid. The volume of the solution is adjusted to 178 ml with demineralized water. 151 ml of pure ethanol are added under stirring. The stirring is maintained for 15 minutes after the end of the addition, and the mixture is then allowed to stay for 10 hours at room temperature.

The resulting precipitate is recovered by centrifugation for 20 minutes at 2,500 RPM. The precipitate is resuspended in 150 ml of pure ethanol, stirred in Ultra-Turrax®, recovered by filtration on fritted Buchner, washed with 300 ml of pure ethanol. ad is finally dried with suction at 40°C for 24 hours .

Thus 5 g of product IC 1772 in the form of white powder are obtained. The product has the following properties:

- SO_3^-	: 3,55 meq/g
- COO^-	: 1,54 meq/g
- S + C	: 5,09 meq/g
- S/C	: 2,31 meq/g

It is practically devoid of N-acetylglucosamine (absence of a signal at ~ 24.5 ppm in the carbon spectrum).

Codex activity	11 ui/mg
APTT activity	9 ui/mg
anti-Xa activity	12 ui/mg

B/ PREPARATION OF O-ACETYLATED HEPARIN FRAGMENT DEVOID OF AFFINITY TO ANTITHROMBIN III (IC 1924)

The product obtained at step A/ is converted to tetrabutylammonium salt by passing through Dowex 50 H^+ resin followed by neutralization with tetrabutylammonium hydroxide. From 9.5 g of the sodium salt, 18 g of the tetrabutylammonium are obtained.

To a solution of 6 g of the resulting salt in dimethylformamide (55 ml) are added after cooling to 0°C acetic anhydride (6.2 ml, 65.6 mmoles) and then triethylamine (9 ml, 65.5 mmoles) and dimethylaminopyridine (403 mg; 3.3 mmoles). After 24 hours, a saturated sodium acetate solution in ethanol is added (250 ml). After centrifugation and washing of the precipitate with ethanol, the solid is desalted in Sephadex G-25 and then subjected to ion exchange by passing through Dowex 50H^+ followed by neutralization with caustic soda. After lyophilization, IC 1924 product is obtained (3 g).

This product has the following properties:

- sulfate/carboxyl ratio: 2.28 meq/g.

The NMR carbon spectrum (methanol 51.6 ppm, i. stnd.) clearly shows that the product is O-acylated. A signal characteristic of C_2 of N-acetylglucosamine at about 56 ppm is absent in the spectrum as for the starting product.

EXAMPLE 19 : PREPARATION OF O-BUTYRYLATED HEPARIN FRAGMENT DEVOID OF AFFINITY TO ANTITHROMBIN III (IC 1925)

6g of tetrabutylammonium salt of IC 1772, obtained as described in Example 18, are butyrylated with butyric anhydride under the conditions described for acetylation. IC 1925 product is obtained (2.96 g) with the following properties:

- sulfate/carboxyl ratio: 2.31 meq/g

^{13}C-NMR spectrum (methanol 51.6 ppm, i. stnd.) contains the signals characteristic of butyl group at15.6, 20.4, and 38.4 ppm.

EXAMPLE 20 : PREPARATION OF O-HEXANOYLATED HEPARIN FRAGMENT DEVOID OF AFFINITY TO ANTITHROMBIN III (IC 1926)

6g of tetrabutylammonium salt of IC 1772, obtained as described in Example 12, are treated with hexanoic anhydride in the same manner as with the acetylation. IC 1926 product is obtained (3g) with the following properties:

- sulfate/carboxyl ratio: 2.20 meq/g

The NMR carbon spectrum (methanol 51.6 ppm, i. stnd.) has the signals characteristic of CH_3 and CH_2 of hexyl group at 15.5, 23.9, 26.2, 32.7 and 36.1 ppm.

The NMR proton spectrum shows the presence of about one hexyl group per disaccharide unit.

EXAMPLE 21 : PREPARATION OF MIXTURE OF FRAGMENTS DEVOID OF AFFINITY TO ANTITHROMBIN III, HOMOGENEOUS IN TERMS OF THEIR MOLECULAR MASS AND O-BUTYRYLATED

A mixture of the fragments devoid of the anticoagulation activity described in Example 12 is fractionated into its various components by gel filtration. Fractions of homogeneous molecular mass are thus obtained with MW of 7700, 6500, 5800, 5300, 4980, 4400, 3900, 3400, 2600, 1860, and 1210.

Example of esterification of a fraction:

A fraction of the mass 2600 (0.20g) is transformed into tributylammonium salt, then lyophilized and dried (0.34g). This product is then dissolved in DMF (2 ml) and then dimethylaminopyridine (18 mg), butyric anhydride (0.49 ml), and tributylamine (0.7ml) are added successively after cooling to 0°C. After 24 hours at room temperature, sodium bicarbonate is added (1 ml of a 5-% solution), then, 2 hours later, the mixture is chromatographed in Sephadex G25 column (1 liter), eluted with 0.2M sodium chloride. The product is lyophilized after desalting, and white clear powder is obtained (0.24 g).

The other fractions are treated in the same manner, and corresponding products are obtained. Their IR analysis reveals the presence of the ester band at 1734 cm^{-1}. The sulfation ratio of the product (the sulfate to carboxyl ratio) does not change after the acylation.

Determination the acylation ratio:

This ratio is determined by chromatography in gaseous phase after butanolysis of the product with a butano – sulfuric acid mixture followed by extraction in chloroform of butyl esters and removal of excessive butanol by washing with water.

EXAMPLE 22 : PREPARATION OF O-ACETYLATED DERMATAN SULFATE (IC 1947)

To a solution of tetrabutylammonium salt of dermatan sulfate (0,91 g) cooled to 0°C, obtained under the same conditions as described for the preparation of tetrabutylammonium heparinate in Example 1, in dimethylformamide (20 ml) are dripped acetic anhydride (1.35 ml, 14.2 mmoles), then triethylamine (1.97 ml, 14.2 mmoles), and dimethylaminopyridine (0.7 mmole). After 24 hours at room temperature, water is added (40 ml), and dialysis is conducted for 72 hours. Sodium salt is prepared by passing through Dowex 50 H^+column at 0°C followed by neutralization with caustic soda. After lyophilization, tan-colored powder is obtained (0.52 g).

O-acetylated dermatan sulfate has the following properties:

- sulfate/carboxyl ratio: 0.99 meq/g (starting product: 1.05 meq/g)
- ^{13}C-NMR spectrum (methanol 51.6 ppm, i. stnd.)
. signal at 25.2 ppm CH_3 of CH_3-CO-NH- (identical to the starting product)
. signal at 23.0 ppm CH_3 of CH_3-CO-O

EXAMPLE 23 : PREPARATION OF O-SUCCINYLATED DERMATAN SULFATE (IC 2020)

Tetrabutylammonium salt of dermatan sulfate (1g) and N,N-dimethylaminopyridine (110 mg) are dissolved in anhydrous dimethylformamide. After addition of succinic anhydride (396 mg) and tributylamine (0.94 ml), the medium is incubated under the anhydrous conditions for 2 hours at 60°C. After cooling and addition of water (2 ml), precipitation is conducted in an icy alcoholic solution saturated with sodium acetate. The precipitate is washed with ethanol, solubilized in apyrogenic water, and then dialyzed with a 5-% sodium bicarbonate for 36 hours and than with water for 3 days. After lyophilization, O-succinylated dermatan sulfate is obtained as sodium salt (0.83 mg).

IR spectrum (KBr) has 1730 cm^{-1} and 1420 cm^{-1} characteristic frequencies (wave number) of carboxylic group.

The NMR carbon spectrum in D_2O (methanol at 51.6 ppm as i. stnd.) is has signals at 33, 34.5 and 183.6 ppm characteristic of succinyl group and a signal at 25.3 ppm characteristic of methyl of acetamido group identical to the initial product. Sulfate/carboxyl ratio is 0.51.

EXAMPLE 24 : PREPARATION OF O-BUTYRYLATED HEPARIN, PRELIMINARILY N-DESULFATED AND N-ACETYLATED (IC 1948)

Heparin is N-desulfated and then N-acetylated using the procedure described by Nagasawa and Inoue (Methods Carbohydr. Chem. Vol. VIII, p.291-294).

Tributylammonium salt (1 g) is obtained by passing through Dowex 50 H^+ resin followed by neutralization with a solution of tributylamine in ethanol.

After lyophilization and drying, this salt is dissolved in dimethylformamide (10 ml) and then, after cooling to 0°C, butyric anhydride (2 ml), tributylamine (2 ml), and dimethylaminopyridine (65 mg) are added. After 24 hours, water (5 ml) is added, and the mixture is then dialyzed with a 5-% solution of sodium bicarbonate and than with water. After [passage through Dowex 50 H^+ followed by neutralization with caustic soda, sodium salt of N-acetylated O-butyrylated heparin is obtained.

The NMR carbon spectrum (methanol 51.6 ppm, i. stnd.) has a signal at 24.6 ppm characteristic of N-acetyl and signals at 16, 20 and 38 ppm characteristic of butyric esters.

This experiment can be repeated with N-acetylated heparin that is partially N-desulfated to obtain a product

which is partially N-desulfated N-acetylated and O-butyrylated.

EXAMPLE 25 : PREPARATION OF PERACETYLATED DERMATAN SULFATE (IC 1950)

Tetrabutylammonium salt of dermatan sulfate (0.8 g) dissolved in dimethylformamide (20 ml) is acetylated by adding dimethylaminopyridine (76 mg), acetic anhydride (1.2 ml), and triethylamine (1.7 ml). The mixture is heated to 80 C for 1 hour.

After cooling to room temperature, water (0.45 ml) is added followed by a 0.3 M solution of sodium acetate in ethanol (100 ml). After centrifugation, the precipitate is dissolved in water and is then dialyzed with distilled water. Sodium salt is obtained by exchange in Dowex 50 H^+ column followed by neutralization with caustic soda. After lyophilization, peracetylated dermatan sulfate is obtained (0.51 g).

This product has the sulfate/carboxyl ratio of 1.07 meq/g (starting product: 1,05 meq/g) and contains about three acetylated groups per one disaccharide unit.

EXAMPLE 26 : PREPARATION OF O-ACETYLATED HEPARIN BENZYL ESTER (IC 1949)

To a solution of tetrabutylammonium heparinate (1 g) in dimethylformamide (10 ml) is added benzyl bromide (0.17 ml). After 24 hours at room temperature, tetrabutylammonium acetate (220 mg) is added. After 24 hours, the resulting benzyl ester is acetylated. For that purpose, dimethylaminopyridine is added (57 mg) followed by triethylamine (1.3 ml) and acetic anhydride (0.9 ml).

After cooling to room temperature, the reaction mixture is stirred for 24 hours. Water is then added, and the product is then precipitated with a saturated solution of sodium acetate in ethanol. After dialysis with distilled water, passage through Dowex 50 H^+, neutralization with caustic soda, and lyophilization, sodium salt of O-acetylated heparin benzyl ester is obtained (0,57 g).

The product has the sulfate/carboxyl ratio of 3.6 meq/g (non-benzylated starting product: 2.20 meq/g).

The carbon spectrum (methanol 51.6 ppm, I. stnd.) has signals at 23.3 ppm (O-acetyl and at 131.6 ppm (benzyl).

The signal of CH_3 of CH_3-CO-NH, at ~ 24.5 ppm is identical to that of the starting product.

EXAMPLE 27 : PREPARATION OF O-ACETYLATED DERMATAN SULFATE BENZYL ESTER (IC 1953)

Tetrabutylammonium salt of dermatan sulfate (1 g) is dissolved in anhydrous dimethylformamide (15 ml). To this solution, cooled to 0°C, is added benzyl bromide (0.25 ml), and the mixture is allowed to stay for 24 hours at room temperature.

Tetrabutylammonium acetate (0,32 g) is then added, and then acetylation is started after 24 hours at room.

Acetic anhydride (1.5 ml) is added followed by triethylamine (2.2 ml) and dimethylaminopyridine (96 mg). After 24 hours, water (0.6 ml) is added, and the product is then precipitated by adding a saturated ethanol solution of sodium acetate.

The product is dialyzed with a 10-% sodium chloride and then with water.

After lyophilization, benzyl ester of O-acetylated dermatan sulfate is obtained (0.63 g).

EXAMPLE 28 : PREPARATION OF O-BUTYRYLATED DERMATAN SULFATE (IC 2018)

Tributylammonium salt of dermatan sulfate (2g), obtained under the same conditions as described for the preparation of tributylammonium heparinate in Example 2, and N,N-dimethylaminopyridine (220 mg) are dissolved in anhydrous dimethylformamide (25 ml). After cooling to 0°C, butyric anhydride (5.9 ml) was dripped followed by tributylamine (8.6 ml). After 24 hours of incubation and after cooling to 0°C, water (1 ml) is added, followed by precipitation in an icy alcoholic solution saturated with sodium acetate. The precipitate is washed with ethanol, solubilized in apyrogenic water, and dialyzed with a 5-% sodium bicarbonate for 36 hours and then with water for 3 days. After lyophilization, sodium salt of O-butyrylated dermatan sulfate is obtained (1.3 g).

The NMR carbon spectrum in D_2O (methanol at 51.6 ppm as i. stnd.) has signals at 15.6, 20.5, and 38.4 ppm characteristic of butyryl group and a signal at 25.5 ppm characteristic of methyl of acetamido group identical to the starting product. The sulfate/carboxyl ratio is 1.05.

EXAMPLE 29: PREPARATION OF O-HEXANOYLATED DERMATAN SULFATE (IC 2019)

Tributylammonium salt of dermatan sulfate (2 g) and N,N-dimethylaminopyridine (220 mg) are dissolved in anhydrous dimethylformamide (25 ml). To this solution, cooled to 0°C, is dripped hexanoic anhydride (9.4 ml) and then tributylamine (8.6 ml). After 24 hours at room temperature and after cooling to 0°C, water is added (1 ml), and precipitation is conducted in a saturated icy solution of sodium acetate. The precipitate is washed with absolute ethanol, solubilized in apyrogenic water, and dialyzed with a 5-% sodium bicarbonate for 36 hours and then with water for 3 days. After lyophilization, sodium salt of O-hexanoylated dermatan sulfate was obtained (1 g).

The NMR spectrum of carbon in D2O (methanol at 15.6 ppm as i. stnd.) has signals at 15.9, 24.2; 26.5, 33.1, and 36.4 ppm characteristic of O-hexanoylated group and a signal at 25.2 ppm characteristic of methyl of acetamido group identical to the starting product. The sulfate/carboxyl ratio is 1.02.

EXAMPLE 30: PROOF OF THE SELECTIVE O-ACETYLATION IN USING THE PROCEDURE ACCORDING TO THE INVENTION AND COMPARISON WITH OTHER ACETYLATION PROCEDURES

The method according to the invention was compared to the procedures disclosed in patents FR 2100735 and EP 256880. More specifically, the properties of the acetic ester of heparin prepared according to the invention (IC 1938, Example 1) were compared with the properties of the acetic ester of heparin obtained by

applying the conditions disclosed in patent FR 2100735 (product A) and the acetic esters of heparin obtained using the procedure disclosed in Examples 3 and 4 of patent EP 256880 (products B and C).

(a) PREPARATION OF PRODUCT A

To a solution of tetrabutylammonium heparinate (1 g) dissolved in anhydrous dimethylformamide (10 ml) is dripped dichlorohexylcarbodiimide (4.2 g) dissolved in dimethylformamide (15 ml) followed by acetic acid (1.16 ml) dissolved in dimethylformamide (25 ml) during 45 minutes at +4°C.

After 24 hours at room temperature, the reaction mixture is filtered and concentrated in vacuum. The residue is suspended in ether. After filtration and washing, the precipitate is dialyzed with distilled water. Sodium salt is obtained by passage through Dowex H^+ resin followed by neutralization with caustic soda. 0.493 g of product A are obtained.

This preparation procedure is also run for 48 hours at +4°C.

(b) PREPARATION DES PRODUCTS B AND C

Products B and C are prepared by acetylation of heparin in a mixture of pyridineformamide with acetyl chloride, using 2 ml of acetyl chloride for product B and 40 ml for product C, under the conditions described in Examples 3 and 4 of patent EP 256880. The acetic ester is then added to water and dialyzed with sodium chloride.

(c) RESULTS

The properties of the products are given in Table 1. The properties of the starting heparin used in each case are given for reference.

TABLE 1

Product	Rapport sulfate / carboxyl (meq/g)	Activity APTT* ui/mg	Activity YW* u/mg
IC 1938	2.28	91	70
Starting heparin	2.20	160	160
A	3.40	42	41
Starting heparin	2.40	160	160
B	2.10	57	39
C	1.15	<2	0.6
Starting heparin	2.40	160	160

APTT and Yin and Wessler activity values are for in vitro measurements.

The results show that, although APTT and YW activity values are lower than those of the starting heparin for all the products, with the greater decrease for products A, B and C, only product IC 1938 retains the sulfate/carboxyl ratio which is practically identical to that of the starting heparin. This results in the fact that the process according to the invention allows for selectively acylating the hydroxyl functions without altering the functional groups of heparin, which is confirmed by the chemical analysis of the products.

Products IC 1938, A, B and C were analyzed for NMR of carbon (methanol 51.6 ppm, i. stnd.). The spectra of these products and the spectra of the starting heparins are given below:

Figure 1 Starting heparin
Figure 2 IC1938
Figure 3 Product A after 24 hours of reaction
Figure 4 Product A after 48 hours of reaction
Figure 5 Product B
Figure 6 Product

The results are as follows:

1. Product IC 1938 has in the carbon spectrum (Figure 2):

- a signal at 23.4 ppm corresponding to CH_3 of CH_3-CO-O
- a signal at 24.4 ppm corresponding to CH_3 of CH_3-CO-, identical to the signal which is present in the starting heparin.

These signals show that amino and carboxyl groups remained, and that there was selective acetylation at the level of hydroxyl groups.

2. Analysis of product A in NMR carbon spectroscopy shows that mainly the product obtained both after 24 hours and after 48 hours (Figures 3 and 4) is a lsourea derivatives of heparin, in which the carboxyl functions are substituted for the group

[insert formula]

because of the use of dicyclohexylcarbodiimide. In fact, the strong signals corresponding to carbon atoms of the above mentioned group can be observed at 27, 24, 54 and 156 ppm, whereas the signal corresponding to CH_3 of CH_3 - CO - 0 at 23 ppm is very weak.

This method does not, therefore, allow for obtaining selective O-acetylation and causes an alteration of the carboxyl functions, which is reflected by an increase in the sulfate/carboxyl ratio.

3. Product B has in its carbon spectrum (Figure 3):

- a signal at 23.1 ppm corresponding to CH_3 of CH_3- CO - O
- a signal at 24.8 ppm corresponding to CH_3 of CH_3- CO - NH, which is clearly stronger than that of the starting heparin and of IC 1938.

These signals show that not only O-acetylation occurs, but there is also strong N-acetylation. The acetylation process that is used is not selective and causes partial N-desulfation followed by acetylation of amines, which also results in the sulfate/carboxyl ratio being decreased.

4. Product C has in its carbon spectrum:

- a signal at 22 ppm corresponding to CH_3 of CH_3-CO-O
- a signal at 24 ppm corresponding to CH_3 of CH_3-CO-NH, which is clearly stronger than that of the starting heparin and of IC 1938.

Similarly to product B, both O- and N-acetylation occur. N-desulfation, which is stronger than in the case of product B, causes a strong decrease in the sulfate/carboxyl ratio.

PHARMACOLOGICAL ACTIVITY OF THE PRODUCTS ACCORDING TO THE INVENTION

A/ Anticoagulation activity in vitro:

1. Evaluation of in vitro activity with reference to gamma-standard:

This measurement is performed with the aid of "sensitized cephalin kaolin time" test. (Diagnostica Stago, Asnières, France) on human plasma. The results are given in Table 2.

TABLE 2

Product	Activity (u/mg)
IC 1940	113
IC 1941	28
IC 1943	7
Heparin	160

IC 1940 = O-butyrylated heparin prepared as described in Example 5

IC 1941 = O-hexanoylated heparin prepared as described in Example 6

IC 1943 - O-decanoylated heparin prepared as described in Example 8

The results obtained show that the products selectively O-acylated according to the invention have the in vitro activity inferior to that of heparin, and their anticoagulation activity decreases as the length of the acylating chain increases.

2. Measurement of the in vitro anticoagulation activity of the products according to the invention on human blood:

The tests are conducted with two doses of the product according to the invention, 2 µg/ml and 4 µg/ml on 5 ml of human blood. The control sample is also taken for each product by replacing the product that is being tested with an isotonic solution of NaCl. After 30 minutes of incubation at room temperature, the blood is centrifuged for 20 minutes at 3000 RPM. The plasma with the platelets removed is decanted for allowing the

following tests to be conducted:
- CKT (cephalin kaolin time) with the aid of "sensitized cephalin kaolin time" kit (Diagnostica Stago, Asnières, France),
- Heptest® (Hemachem, St. Louis, USA). The results are given in Table 3. Each result corresponds to the average of three tests.

TABLE 3

Product	Dose (µg/ml)	CKT (S)	HEPTEST (S)
Control		48.00 ± 2.70	21.50 ± 1.73
IC 1940	2	208.75 ± 45.54	61.00 ± 8.52
IC 1940	4	428.00 ± 103.43	101.00 ± 28.36
Control		45.00 ± 5.00	23.66 ± 2.08
IC 1941	2	53.00 ± 5.19	40.33 ± 7.63
IC 1941	4	84.66 ± 15.01	61.33 ± 11.01
Control		49.66 ± 8.50	23.00 ± 4.35
IC 1943	2	50.00 ± 9.54	23.00 ± 4.58
IC 1943	4	56.00 ± 10.00	24.00 ± 5.29

For the two methods, the results show a longer coagulation time for products IC 1940 and IC 1941. This time increase is proportional to the dose.

At the same time, with these methods in vitro, product IC 1943 showed but a very slight effect on the increase in the coagulation time.

3. Measurement of the anticoagulation effect in vitro of the products of the invention by thromboelastography on human blood:

The products according to the invention in the doses of 2 µg/ml and 4 µg/ml were used for 5 ml of human blood as described in the preceding tests, with a control test tube for each product containing instead an isotonic solution of NaCl.

After 30 minutes of incubation at an ambient temperature, thromboelastographic recording was conducted with the aid of Hellige thromboelastograph using 0.25 ml of enriched with 0.1 ml of 0.058M $CaCl_2$.

The results are given in Table 4.

TABLE 4

Product	Dose (µg/ml)	r *	k *	r + k *	amx *	IPT *
Control		13,00 ± 0,81	8,25 ± 1,25	21,25 ± 1,50	48,00 ± 2,44	11,25 ± 2,36
IC 1940	2	33,50 ± 2,88	22,25 ± 4,03	55,75 ± 5,85	35,50 ± 3,69	2,57 ± 0,77
IC 1940	4	78,75 ± 17,34	44,00 ± 8,68	122,75 ± 13,02	27,00 ± 16	0,82 ± 0,27
Control		10,50 ± 0,70	4,50 ± 2,12	15,00 ± 2,82	57,00 ± 8,48	37,00 ± 28,28
IC 1941	2	14,00 ± 3,00	10,33 ± 6,50	24,33 ± 6,00	51,66 ± 10,96	21,33 ± 19,85
IC 1941	4	20,33 ± 3,51	15,00 ± 4,58	35,33 ± 5,03	40,33 ± 2,51	4,66 ± 2,08
Control		13,33 ± 2,30	9,00 ± 2,64	22,33 ± 4,93	47,00 ± 4,58	10,33 ± 4,04
IG 1943	2	12,66 ± 2,08	8.00 ± 2,64	20,66 ± 4,72	47,00 ± 5,29	12,00 ± 5,29
IC 1943	4	12.66 ± 2,08	9,83 ± 3,01	22,50 ± 5,07	45,66 ± 5,77	9,33 ± 4,04

* r = reaction time
k = coagulation time corresponding to the amplitude of the record of 20 mm
amx = maximum amplitude
IPT = index of the thrombodynamic potential

The results show that IC 1940 and IC 1941 cause an increase in r+ k and a decrease in amx and IPT, which corresponds to the increased hypocoagulation. This hypocoagulation increases both with the dose used and with the length of the acylating chain.

IC 1943 did not cause any remarkable modification of parameters in these tests.

B/ IN VIVO ANTICOAGULATION ACTIVITY:

1. Measurement of the anticoagulation activity in vivo for the products according to the invention in rabbits (IV administration) :

The tests are conducted on New Zealand male rabbits. Blood is taken at the level of the median artery of the

ear before injection of the product. A solution of the product being tested – 25 mg in 5 ml of isotonic NaCl solute into the marginal vein.

CKT and HEPTEST^{R} tests are conducted with the blood samples taken before the product is injected and with the samples taken 6 h, 24 h, 48 h and 96 h after the injection.

The results are given in Figures 7 and 8. They show a clear increase in the time of the anticoagulation activity, and this increase becomes greater with an increase in the length of the acylating chain.

In fact, the anticoagulation activity of IC 1940 product is evident more 6 hours after the injection, whereas this activity is evident 24 hours after the injection of IC 1941 product and is extended up to 96 hours for IC 1943.

2. Measurement of <u>in vivo</u> anticoagulation activity of the products of the invention by thromboelastography:

The products that are being tested are injected intravenously as described in the previous test (25 mg in 5 ml of isotonic NaCl solute). The thromboelastographic recording is carried out with the aid of Hellige thromboelastograph with samples of 0.25 ml of blood taken 6 h, 24 h, 48 h et 96 h after the injection, and the supplemental samples are taken when the anticoagulation activity was observed after 96 hours .

The results are shown in Tables 5, 6 and 7.

TABLE 5
PRODUCT TESTED : IC 1940

	r	k	r + k	amx	IPT
Before injection	13	6	19	67	33
6 h. after the injection	91	60	151	56	2,1
24 h. after the injection	12	5	17	67	40
96 h. after the injection	8	3	11	70	77

TABLE 6
PRODUCT TESTED : IC 1941

	r	k	r + k	amx	IPT
Before injection	13	5	18	67	40
6 h. after the injection	135	31	166	45	2,6
24 h. after the injection	19	12	31	58	11,5
48 h. after the injection	22	10	32	72	25

TABLE 7
PRODUCT TESTED : IC 1943

	R	k	r + k	amx	IPT
Before injection	12	4	16	69	55
6 h. after the injection	40	27	67	>40	-
24 h. after the injection	13	7	20	72	36
48 h. after the injection	53	34	87	54,5	4,7
72 h. after the injection	112	185	297	> 38	-
96 h. after the injection	82	45	127	62	3,6
168 h. after the injection	19	9	28	68	23
192 h. after the injection	17	5	22	70	46

The results obtained show that for all the products, there is a strong increase in r + k and a decrease in IPT after 6 hours, which indicates to an increase in the hypocoagulation ability. This can be extended up to at least 24 hours after the injection for IC 1941, and it can still be measured 96 hours after the injection for IC 1943.

Thus it can be concluded that all the products have a strong anticoagulation activity in vivo, that this activity is extended in time, and that IC 1943, which had a very small activity if any in in vitro tests, showed a very high activity and extended time in the tests in vivo.

3. The anticoagulation activity of products IC 1945, IC 1957 et IC 1958 prepared according to Examples 12, 13 et 14, respectively, as tested in vivo in rabbits.

The results showed the prolonged activity with intravenous administration and with subcutaneous administration.

C/ ACTIVITY IN IN VITRO MODEL IN INHIBITING HIV 1 AND 2 VIRUS:

The products prepared according to the invention were tested in a model for inhibiting HIV-1 and HIV-2 viruses in vitro as described in R. Pauwels et al, J. Virol. Methods, 1987, 16, 171-185.

All the products tested proved active in doses that were totally devoid of cytotoxicity. More specifically, it has been concluded that IC 1925 and IC 1926 products were more active than the starting products.

D/ ACTIVITY IN IN VITRO MODEL FOR INHIBITING THE FORMATION SYNCYTIA

Syncytia are giant cells with multiple nuclei8 formed by fusion of sound T4 lymphocytes with infected T4 lymphocytes.

The products prepared according to the invention were tested in a model of co-culture of MOLT4 cells (sound T4 lymphocytes) and HUT-78/HTLV III B cells (infected T4 lymphocytes of the human line).

All the products tested proved active in this model in doses that were totally devoid of cytotoxicity. More specifically, it has been concluded that IC 1924, IC 1925 and IC 1926 products were more active than the starting products.

E/ ACTIVITY IN IN VITRO MODEL FOR INHIBITING ENVELOPED DIFFERENT VIRUSES

The activity of the products prepared according to the invention with respect to inhibiting different ADN or ARN enveloped viruses, more specifically, for the following viruses:

- HSV 1 and 2 (herpes simplex virus 1 and 2, virus at ADN)
- VSV (vesicular stomatitis virus) Virus at ARN
- Sindbis virus Virus at ARN

All the products tested proved active in inhibiting these viruses in doses devoid of cytotoxicity. More specifically, IC 1924, IC 1925 and IC 1926 products had the activity that was very strong against VSV and Sindbis virus compared to the starting product.

F/ ACTIVITY IN IN VIVO MODEL FOR INHIBITING PROLIFERATION OF SMOOTH MUSCULAR CELLS

The products prepared according to the invention were tested in a model for inhibiting proliferation of smooth muscular cells in vivo in rats (model of the balloon catheter as described in A.W. Clowes and M.M. Clowes, Labor. Investig., 1985, 52 (6), 612-616.

The products tested proved active. More specifically, IC 1924, IC 1925 and IC 1926 products had activity that was greater than that of the starting product.

Claims

1. Selectively O-acylated glycosaminoglycans of the following formula (I):

in which:

- G is a group (a) of the formula

or a group (a') of the formula:

or a group (b) of the formula:

-U is a group (c) of the formula:

or a group (d) of the formula:

or a radical of group (c) or group (d) after the cyclic oxidation followed by β-elimination or acid hydrolysis;

- A is a group R_1, a group R_1-(c), a group R_1 - (d), or a group (e) of the formula:

or a radical of group (c) or group (d) after the cyclic oxidation followed by β-elimination or acid hydrolysis;

- B is a group O-R_1, a group (a)-OR_1, a group (a')-OR_1, a group (b)-OR_1,a group (f) of the formula:

or a group (g) de formula:

or a group (a), a group (a'), or a group (b) to which is attached a radical of (c) or (d) such as is present after the cyclic oxidation followed by β-elimination or acid hydrolysis;

- R_1 is H, SO_3^- or an acyl, the acyl being a residue of a not α,β-unsatrurated carboxylic acid or dicarboxylic acid selected from:

. an alkanoyl group having 1 to 18 carbon atoms;

. an alkanoyl group having 2 to 3 carbon atoms substituted with:

- a cycloalkyl group having 3 to 7 carbon atoms,
- a phenyl group eventually substituted with one or several alkyl radicals having1 to 14 carbon atom, halogen atoms, or NO_2 or OCH_3 group,
- a unsaturated aliphatic hydrocarbon radical having 4 to 16 carbon atom;

. a benzoyl group eventually substituted with one of several alkyl radicals having 1 to 4 carbon atoms, halogen atoms, or NO_2 or OCH_3 groups;

. a cycloalkyl group (3-7 C) carbonyl;

- R_2 is SO_3^- and/or an acetyl radical, provided that the ratio of N-acetyl glucosamine is at most equal to that of heparin when R_1 is an acetyl radical;
- R_3 is a hydrogen atom or an alkyl radical having 1 to 10 carbon atoms, preferably 1 to 4 carbon atoms, or a phenyl-alkyl radical having 7 to12 carbon atoms, or an alkali or alkali-earth metal cation;
- n is an integer from 1 to 80,

R_1 being acyl in the ratio of at least 0.1 to 3 acyl groups per disaccharide unit, preferably, 0.5 to 2 acyl groups, and their pharmaceutically acceptable salts.

2. Selectively O-acylated glycosaminoglycans of claim 1, characterized by the fact that they have the following formula:

in which:

- A has the meaning defined in claim 1;
- R_1 is H and/or SO_3^- and/or an acyl group such as defined in formula (I) ;
- R_2 is SO_3^- and/or an acetyl radical, provided that the ratio of N-acetyl glucosamine is at most equal to that of heparin when R_1 is an acetyl radical;
- R3 is a hydrogen atom, an alkyl radical having 1 to 10 carbon atoms, preferably 1 to 4 carbon atoms, a phenyl-alkyl radical having 7 to 12 carbon atoms, or an alkali or alkali-earth metal cation;
- B is (a) -OR_1 or (f), (a) and (f) being such as defined in claim 1, or OR1, or group (a) to which is attached a residue of (c) r (d) such as present after the cyclic oxidation followed by β-elimination or acid hydrolysis;
- n is an integer from 1 to 80, provided that when A is R_1, a group R_1-(c) or a group R_1-(d) and B is O-R_1, a group (a)-OR_1 or a group (b) –OR_1, n is an integer from 1 to 16.

3. Selectively O-acylated glycosaminoglycans of claim 2, characterized by the fact that they have the following formula:

in which:
- A, B, R_1, R_2 and n have the meanings defined in claim 2;
- R_3 is a hydrogen atom or an alkali or alkali-earth metal cation.

4. Selectively O-acylated glycosaminoglycans of claim 2, characterized by the fact that they have the following formula:

in which:
- A, B, R_1, R_2 and n have the meanings defined in claim 2;;
- R_3 is an alkyl radical having 1 to 10 carbon atoms, preferably from 1 to 4 carbon atoms, or a phenyl-alkyl radical having from 7 to 12 carbon atoms.

5. Selectively O-acylated glycosaminoglycans of claim 1, characterized by the fact that they have the following formula (II):

in which:
- A has the meaning defined in claim 1,
- R_1 is H and/or SO_3^- and/or and acyl group, a residue of a not α,β-unsatrurated carboxylic acid or dicarboxylic acid selected from:
. an alkanoyl group having 4 to 18 carbon atoms,
. an alkanoyl group having 2 or 3 carbon atoms substituted with:
- a cycloalkyl group having 3 to 7 carbon atoms,
- a phenyl group eventually substituted with one or several alkyl radicals having 1 to 14 carbon atoms, halogen atoms or NO_2 or OCH_3 group,
- an unsaturated aliphatic hydrocarbon radical having 4 to 16 carbon atoms,
. a benzoyl group eventually substituted with one or several alkyl radicals having 1 to 4 carbon atoms, halogen atoms or NO_2 or OCH_3 group,
. a cycloalkyl group (3-7C) carbonyl;
- R_2 is SO_3 and/or an acetyl radical, provided that the ratio of N-acetyl glucosamine is at most equal to that of heparin when R_1 is an acetyl radical;
- R_3 is a hydrogen atom or an alkyl radical having 1 to 10 carbon atoms, preferably 1 to 4 carbon atoms, or a phenyl-alkyl radical having 7 to 12 carbon atoms, or an alkali or alkali-earth metal cation;
- n is an integer from 1 to 80,
R_1 being acyl in the ratio of at least, 0.5 to 2 acyl groups preferably 1 acyl group.

6. Selectively O-acylated glycosaminoglycans of claim 5, characterized by the fact that they have the following formula II:

in which:
- A, B, R_1, R_2 and n have the meanings defined in claim 5,
- R_3 is a hydrogen atom or an alkali or alkali-earth metal cation.

7. Selectively O-acylated glycosaminoglycans of claim 5, characterized by the fact that they have the following formula II:

in which:
- A, B, R_1, R_2 and n have the meanings defined in claim 5,
- R_3 is an alkyl radical having 1 to 10 carbon atoms, preferably 1 to 4 carbon atoms, or a phenyl-alkyl radical having 7 to 12 carbon atoms.

8. Selectively O-acylated glycosaminoglycans of any of claims 1 through 4, characterized by the fact that glycosaminoglycan is selected from the group consisting of a mixture of fragments of heparin having a molecular mass below 10,000 Daltons, a mixture fragments of heparin having a mean molecular mass between 2,000 and 7,000 Daltons, a mixture fragments of heparin having a mean molecular mass of about 4,500 Daltons, a mixture fragments of heparin having a mean molecular mass of about 2,500 Daltons, a mixture fragments of heparin having which is homogeneous in terms of its molecular mass, a heparin fragment obtained by synthesis, homogeneous in terms of its molecular mass with regard to its functioning, the acyl group being used at a ratio of at least 0.1 to 3 groups per disaccharide unit, preferably 0.5 to 2 acyl groups.

9. Selectively O-acylated glycosaminoglycans of any of claims 5 through 7, characterized by the fact that glycosaminoglycan is heparin, an acyl group being in a ratio of at least 0.5 to 2 groups per disaccharide unit, preferably 1 acyl group.

10. Selectively O-acylated glycosaminoglycans of claims 1 through 8, characterized by the fact that they have the following formula III:

in which:
- A is R_1, or R_1-(c) or R_1-(d) as defined, in claim 1;
- R_1 is H, and/or SO_3^- and/or an acyl group as defined in claim 1;
- R_2 is SO_3^- and/or an acetyl radical, provided that the ratio of N-acetyl glucosamine is at most equal to that of heparin when R_1 is an acetyl radical;
- R_3 is a hydrogen atom or an alkyl radical having 1 to 10 carbon atoms, preferably 1 to 4 carbon atoms, or a phenyl-alkyl radical having 7 to12 carbon atoms, or an alkali or alkali-earth metal cation;
- n is an integer from 3 to 12.

11. Selectively O-acylated glycosaminoglycans of claims 1 through 8, characterized by the fact that they have the following formula IV:

in which:
- R_1 is H, and/or SO_3^- and/or an acyl group as defined in claim 1;
- R_2 is SO_3^- and/or an acetyl radical, provided that the ratio of N-acetyl glucosamine is at most equal to that of heparin when R_1 is an acetyl radical;
- R_3 is a hydrogen atom or an alkyl radical having 1 to 10 carbon atoms, preferably 1 to 4 carbon atoms, or a phenyl-alkyl radical having 7 to12 carbon atoms, or an alkali or alkali-earth metal cation;
- B is (a)-OR_1 as defined in claim 1 or OR_1 ;
- n is an integer from 2 to 20.

12. Selectively O-acylated glycosaminoglycans of claims 1 through 4, characterized by the fact that glycosaminoglycan is selected from heparin, a fraction or a fragment of heparin devoid of the site for fixation of antithrombin III.

13. Selectively O-acylated glycosaminoglycans of any claims 1, 2, 5 and 12, characterized by the fact that they have the following formula V:

in which:
- A is R_1-(c), R_1-(d) or a residue of (c) or (d) after the cyclic oxidation followed by β-elimination or acid hydrolysis;
- B is (a) $-OR_1$ or a group (a) to which is attached a residue of (c) or (d) such as present after the cyclic oxidation followed by β-elimination or acid hydrolysis;
- R_1 has the meaning defined in claim 1;
- R_2 is SO_3^- or an acetyl radical, with the ratio of SO_3^- being of about 90%;
- R_3 is a hydrogen atom or an alkali or alkali-earth metal cation;
- n is an integer from 1 to 80.

14. Selectively O-acylated glycosaminoglycans of any claims 1, 2, 5 and 12, characterized by the fact that they have the following formula VI:

in which:
- A is R_1, R_1-(c), R_1-(d), or a residue of (c), or (d) after the cyclic oxidation followed by β-elimination;
- U is:

or, with one member per at least two chains, a non-sulfated uronic acid (D-glucuronic or L-iduronic) open between the carbon atoms at positions 2 and 3, of the formula:

- B is (a) $-OR_1$ or OR_1, or group (a) to which is attached a residue of (c) or (d) such as present in after the cyclic oxidation followed by β-elimination;
- R_1 has the meaning defined in claim 1;
- R_2 is SO_3^- or an acetyl radical, with the ratio of SO_3^- of about 90%;
- R_3 is a hydrogen atom or an alkali or alkali-earth metal cation;
- n is an integer from 2 to 18.

15. Selectively O-acylated glycosaminoglycans of any claims 1, 2, 5 and 12, characterized by the fact that they have the following formula VI:
in which:
- n is an integer from 7 to 15 for majority species,
- R_1 is an alkanoyl radical having 2 to 10 carbon atoms, advantageously 4 to 10, preferably 4 to 6 carbon atoms.

16. Selectively O-acylated glycosaminoglycans of claims 14 and 15, characterized by the fact that they are composed of a mixture of fragments homogeneous with regard to the molecular mass, in which n is an integer from 2 to 12.

17. Selectively O-acylated glycosaminoglycans of any claims 1, 2, 5 and 12, characterized by the fact that they have the following formula VII:

in which:
- A is R_1, R_1-(c), or R_1-(d) as defined in formula (I) ;
- R_1 is an alkanoyl radical having 2 to 18 carbon atoms;
- R_3 is a hydrogen atom or an alkali or alkali-earth metal cation;
- B is (a) $-OR_1$ as defined in formula (I) or OR_1 ;
- n is an integer from 1 to 80.

18. Selectively O-acylated glycosaminoglycans of claims 1, characterized by the fact that they have the following formula VIII:

in which:
- A has the meaning defined in claim 1;
- R_1 is H and/or SO_3^- and/or an acyl group as defined in claim 1;
- R_3 is a hydrogen atom, an alkyl radical having 1 to 10 carbon atoms, preferably 1 to 4 carbon atoms, or a phenyl-alkyl radical having 7 to 12 carbon atoms, or an alkali or alkali-earth metal cation;
- B is (b) $-OR_1$ or (g) as defined in formula (I), or OR_1, or group (b) to which is attached a residue of (c) or (d) such as present after the cyclic oxidation followed by β-elimination or acid hydrolysis;
- n is an integer from 1 to 80;

19. Selectively O-acylated glycosaminoglycans of claims 18, characterized by the fact that they have the following formula VIII:

In which:
- A, B, R_1 and n have the meanings defined in claim 18;
- R_3 is a hydrogen atom or an alkali or alkali-earth metal cation.

20. Selectively O-acylated glycosaminoglycans of claims 18, characterized by the fact that they have the following formula VIII:

in which:
- A, B, R_1 and n have the meanings defined in claim 18;
- R_3 is an alkyl radical having 1 to 10 carbon atoms, preferably 1 to 4 carbon atoms, or a phenyl-alkyl radical having 7 to 12 carbon atoms.

21. Selectively O-acylated glycosaminoglycans of any claims 1, 8, 19 and 20, characterized by the fact that glycosaminoglycan is selected from the group consisting of dermatan sulfate and its fragments, or chondroitin-4 sulfate and chondroitin-6-sulfate and their fragments, the acyl group being in a ratio of at least 0.1 to 3 acyl groups per disaccharide unit, preferably 0.5 to 2 acyl groups.

22. Selectively O-acylated glycosaminoglycans of any claims 1 through 21, wherein R_1 is an alkanoyl radical having 4 to 10 carbon atoms.

23. A process for the preparation of selectively O-acylated glycosaminoglycans of any of claims 1 to 22, characterized by (1) transforming (1) a glycosaminoglycan of the following formula IX:

in which:
- G° is a group (a)° of the formula:

or a group (a')° of the formula:

or a group (b)° of the formula:

- U° is a group (c)° of the formula:

or a group (d)° of the formula:

or a residue from group (c)° or group (d)° after the cyclic oxidation followed by β-elimination or acid hydrolysis;

-A° is a group R_1°, a group R_1°-(c)°, a group R_1°-(d)°, or a group (e)° of formula:

or a residue of the group (c)° or group (d)° after the cyclic oxidation followed by β-elimination or acid hydrolysis;

- B° is a group OR_1°, a group (a)°-OR_1°, a group (a')-OR_1°, a group (b)°-OR_1°, or a group (f)° of the formula:

or a group (g)° of the formula:

or groups (a)°, (a')°, or (b)° to which is attached a residue of (c)° or (d)° such as present after the cyclic oxidation followed by β-elimination or acid hydrolysis;

- R_1° is H or SO_3^-
- R_2 is SO_3^- or an acetyl radical, provided that the ratio of N-acetyl glucosamine is at most equal to that of heparin when R_1 is an acetyl radical;
- R_3 is a hydrocarbon atom, or an alkyl radical having 1 to 10 carbon atoms, or a phenyl-alkyl radical having 7 to 12 carbon atoms, or an alkali or alkali-metal cation;
- n is an integer from 1 to 80,

and a salt of said glycosaminoglycan soluble in an organic aprotic polar solvent;

(2) treating said salt with an anhydride of the formula:

Acyl — O — Acyl

in which Acyl is as defined in claim 1 in said organic aprotic polar solvent in the presence of the catalytic quantities of pyridine or a dialkylaminopyridine and a proton acceptor;

(3) precipitating the resulting product by acting with a solution of sodium acetate in ethanol, and

(4) isolating the selectively O-acylated glycosaminoglycan by dissolving the resulting precipitate in water and by conducting dialysis, and eventually transforming the resulting sodium salt of selectively O-acylated glycosaminoglycan to another pharmaceutically acceptable salt.

24. The process of claim 24 *[sic]*, characterized by the fact that the glycosaminoglycan that is used at step (1) is selected from glycosaminoglycan is selected from the group consisting of a mixture of fragments of heparin having a molecular mass below 10,000 Daltons, a mixture fragments of heparin having a mean molecular mass between 2,000 and 7,000 Daltons, a mixture fragments of heparin having a mean molecular mass of about 4,500 Daltons, a mixture fragments of heparin having a mean molecular mass of about 2,500 Daltons, a mixture fragments of heparin having which is homogeneous in terms of its molecular mass, a heparin fragment obtained by synthesis, homogeneous in terms of its molecular mass with regard to its functioning.

25. The process of claim 23, characterized by the fact that the glycosaminoglycan that is used at step (1) is heparin or a fraction or a fragment of heparin devoid of a site for de fixation of antithrombin III.

26. The process of claim 23, characterized by the fact that the glycosaminoglycan that is used at step (1) is selected from the group consisting of dermatan sulfate and its fragments or chondrotin-4 sulfate and chondrotin-6 sulfate and their fragments.

27. The process of any of claim 23 through 26, characterized by the fact that the glycosaminoglycan that is used at step (1) is as tertiary amine salt, more specifically a tributylamine salt or a quaternary amine salt, more specifically a tetrabutylammonium salt.

28. The process of any of claim 23 through 27, characterized by the fact that the anhydride that is used at step (2) is an anhydride of an alkanoic acid having 2 to 10 carbon atoms, advantageously 4 to 10, preferably 4 or 6 carbon atoms.

29. The process of any of claim 23 through 28, characterized by the fact that step (2) is carried out in an aprotic polar solvent selected from the group consisting of dimethylformamide, hexamethylphosphorotriamide, pyridine, or a mixture of these solvents or their mixture with dichloromethane.

30. The process of any of claim 23 through 29, characterized by the fact that the proton acceptor for step (2) is a substance selected from the group consisting of pyridine, triethylamine, and tributylamine.

31. The process of any of claim 23 through 30, characterized by the fact that the dialysis of step (4) is carried out in the presence of a weak base.

32. The process of any of claim 23 through 31, characterized by the fact that step (2) is carried out at a temperature between 0°C and 100°C, more specifically between 50°C and 100°C.

33. A pharmaceutical composition, characterized by the fact that is comprises as an active substance an effective quantity of at least one selectively O-acylated glycosaminoglycan of claims 1 through 22 with a pharmaceutical carrier.

34. The pharmaceutical composition of claim 33, characterized by the fact that the selectively O-acylated glycosaminoglycan is in the form of a pharmaceutically acceptable sodium, magnesium or calcium.

35. The pharmaceutical composition of claims 32 and 34, characterized by the fact that the pharmaceutical carrier is adapted for oral administration and is in the form of gastrically resistant gel capsules, tablets, pills or in the form of drinking solutes containing advantageously 50 mg to 5 g per dose, preferably 100 to 1000 mg per gel capsule, tablet or pill and 10 to 150 mg drinking solutes.

36. The pharmaceutical composition of claims 33 and 34, characterized by the fact that it is in the form of a solution of injections, sterile or sterilizable, for intravenous, intramuscular or subcutaneous administration, the solution containing advantageously 50 to 200mg/ml of selectively O-acylated glycosaminoglycan for subcutaneous injections or 20 to 200 mg/ml of selectively O-acylated glycosaminoglycan for intravenous injections or perfusion.

37. A pharmaceutical composition of any claims 33 through 36 for treatment or prevention of diseases cased by enveloped viruses.

38. The pharmaceutical composition of claim 36 for treatment or prevention of diseases caused by retroviruses such as SIDA.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

according to Rule 45 of the European Patent Patent Convention is regarded, for the purposes of further procedure as the European Patent Search

Application No.

EP 89 40 2086

DOCUMENTS REGARDED AS PERTINENT

Category	Citation of documents with indication of the pertinent parts	Ref. to Claims	CLASS INDEX OF APPLICATION (Int. Cl. 5)
X	CHEMICAL ABSTRACTS OF JAPAN , vol. 75, 1971, page 100, abstract No. 7728a, Columbus, Ohio, US; & JP-A-71 09 327 (SUMITOMO CHEMICAL CO. LTD.) 03-09-1971 * Resume *	1	C 08 B 37/10 C 08 B 37/08
Y	FR-A-2 584 728 (CHOAY) * Claims *		
Y	US-A-4 331 697 (KUDO et al.) * Column 4, lines 60 – 66; column 5, lines 1 – 20; claims *		
			FIELD OF SEARCH (Int. Cl. 5)
			C 08 B

Search Office	Search Date	Examiner
HAGUE	11 - 03, 1989	LENSEN, H. W. M.

FIGURE 7

1 ANTICOAGULATION ACTIVITY AS MEASURED BY TCK (SECONDS)

2 TIME AFTER INJECTION (HOURS)

FIG. 8

1. ANTICOAGULATION ACTIVITY AS MEASURED BY HEPTEST (SECONDS)

2 TIME AFTER INJECTION (HOURS)